

FIGURE 1A

1 CGGATGCTGC TGCTACTGTC ACTTCTGCCG CTGCCGCTGT TGTTACAGAT
 51 TTTGCTTTTG CTCCTTCTAC CGCATGACAA TTGTTTTCTT CGCCTAAGCA
 101 GATACCAGCC TCAGATGCTC AAGGTGAGAG TCTTGCCTTT CGCTCTGGGC
 151 TATTGGTTCA CTTAATCCGG TCAATTTGTT CGCTGCTCGT GGTGTCTTT
 201 CTCCCCGCCC TCCTTCCCCC TGTTTTGTTT TGTTTCGCTT GCTTTCGGGG
 251 GGACGCTCCT TCCCTCAGTC AGAAGAGCTG GAATTGCTTG AGAGGCGTAT
 301 AAGGAATTAT AAAAGTGGCC AGGAAACACG AGCGCAGTGA CTGCAGAGCT
 351 GCCCTTGGCT TCGGCAAGGC AGCGTGAGCG GCAGAGGGCT CGGGCAGGGG
 401 GCGGGGGGTC TCCTTTTTCC CGTGCGTTCC TCTTCTCCCA GTTCGGATGA
 451 TGTTGCTGTT TCGGACCTCT CGCTGACTCC TGCCCTGTGA TTTTGTCTGA
 501 GCGCTGTGAC TGTTACTCCG TCTCTTTCTG TCTGTGTTTC ACAGTAATGG
 551 ACTGTGATAG AGTTAAGGCC TTTTGGAGGT GAGCTGTGTC ACAGCTGATG
 601 CTTAAACATG TCTGAAGTAG GCACCGAGAC TTTCCCCAGC CCCTCGGCTC
 651 AGCTGAGCCC TGATGCATCC CTTGGCGGGC TCCCGGCTGA GGAGAACATG
 701 CCGGGGCCCC ACAGAGAGGA CAGCAGGGTC CCAGGTGTGG CAGGCCTGGC
 751 CTCGACCTGC TCGGTGTGCC TGGAAGCAGA GCGACTGAAG GGCTGCCTCA
 801 ACTCTGAGAA GATCTGCATC GCCCCTATCC TGGCTTGCCT GCTCAGCCTC
 851 TGCCTCTGCA TTGCTGGCCT CAAGTGGGTC TTTGTGGACA AGATTTTGA
 901 GTATGACTCT CCTACACACC TTGACCCTGG GAGGATAGGA CAAGACCCAA
 951 GGAGCACTGT GGATCCTACA GCTCTGTCTG CCTGGGTGCC TTCGGAGGTG
 1001 TATGCCTCAC CCTTCCCCAT ACCTAGCCTT GAGAGCAAGG CTGAAGTGAC
 1051 AGTGCAAAC TACAGCTCGC TCGTGCCCTC CAGGCCCTTC CTTCAGCCTT
 1101 CTCTCTACAA CCGCATCCTA GATGTCGGGT TGTGGTCCTC TGCCACACCG
 1151 TCACTGTCAC CATCCTCCCT GGAGCCTACC ACGGCATCTC AGGCACAAGC
 1201 AACAGAAACC AATCTCCAAA CTGCTCCAAA ACTTTCCACT TCTACATCTA
 1251 CAACTGGGAC AAGTCATCTC ACAAATGTG ACATAAAGCA GAAAGCCTTC
 1301 TGTGTAAATG GGGGAGAGTG CTACATGGTT AAAGACCTCC CAAACCCTCC

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FIGURE 1B

| | | | | | |
|------|------------|------------|------------|------------|------------|
| 1351 | ACGATACCTA | TGCAGGTGCC | CAAATGAATT | TACTGGTGAT | CGCTGCCAAA |
| 1401 | ACTACGTAAT | GGCCAGCTTC | TACAAGCATC | TTGGGATTGA | ATTTATGGAA |
| 1451 | GCTGAGGAAC | TGTACCAGAA | ACGGGTGCTG | ACCATAACTG | GCATTTGCAT |
| 1501 | TGCTCTTCTA | GTAGTTGGCA | TCATGTGTGT | GGTGGCCTAC | TGCAAAACCA |
| 1551 | AGAAGCAGAG | GAAAAAGTTG | CATGACCGCC | TTCGGCAGAG | CCTTCGCTCA |
| 1601 | GAGAGGAACA | ACGTTATGAA | CATGGCAAAT | GGGCCACACC | ACCCCAACCC |
| 1651 | ACCACCAGAC | AATGTCCAGC | TGGTGAATCA | GTACGTTTCA | AAAAACATAA |
| 1701 | TCTCCAGTGA | ACGTGTCGTT | GAGCGAGAAA | CCGAGACCTC | GTTTTCCACA |
| 1751 | AGCCACTACA | CCTCAACAAC | TCATCACTCC | ATGACAGTCA | CCCAGACGCC |
| 1801 | TAGCCACAGC | TGGAGTAATG | GCCATACCGA | AAGCATTCTC | TCCGAAAGCC |
| 1851 | ACTCCGTGCT | CSTCAGCTCC | TCAGTGGAGA | ATAGCAGGCA | CACCAGCCCA |
| 1901 | ACAGGGCCAC | GAGGCCGCCT | CAATGGCATT | GGTGGGCCAA | GGGAAGGCAA |
| 1951 | CAGCTTCCTC | CGGCATGCAA | GAGAGACCCC | TGACTCCTAC | CGAGACTCTC |
| 2001 | CTCACAGTGA | AAGGTATGTC | TCAGCTATGA | CCACACCAGC | TCGCATGTCA |
| 2051 | CCCGTTGATT | TCCACACTCC | AACTTCTCCC | AAGTCCCCTC | CATCTGAAAT |
| 2101 | GTCACCACCA | GTTTCCAGCT | TGACCATCTC | CATCCCTTCG | GTGGCGGTGA |
| 2151 | GTCCCTTTAT | GGACGAGGAG | AGACCGCTGC | TGTTGGTGAC | CCCACCACGG |
| 2201 | CTGCGTGAGA | AGTACGACAA | CCACCTTCAG | CAATTCAACT | CCTTCCACAA |
| 2251 | CAATCCCACC | CATGAGAGCA | ACAGTCTGCC | ACCCAGTCCT | CTGAGGATAG |
| 2301 | TGGAGGATGA | AGAGTATGAG | ACCACGCAGG | AGTACGAACC | AGCACAGGAG |
| 2351 | CCTCCAAAGA | AACTCACCAA | CAGCCGGAGG | GTGAAAAGAA | CAAAGCCCAA |
| 2401 | TGGCCATATT | TCCAGCAGGG | TAGAAGTGGA | CTCCGACACA | AGCTCTCAGA |
| 2451 | GCACTAGCTC | TGAGAGCGAA | ACAGAAGATG | AAAGAATAGG | TGAGGATACA |
| 2501 | CCATTTCTTA | GCATACAAAA | TCCCATGGCA | ACCAGTCTGG | AGCCAGCCGC |
| 2551 | TGCATATCGG | CTGGCTGAGA | ACAGGACTAA | CCCGGCAAAT | CGCTTCTCCA |
| 2601 | CACCAGAAGA | GTTGCAAGCA | AGGTTGTCCA | GTGTAATAGC | TAACCAAGAC |
| 2651 | CCTATTGCTG | TATAAGACAT | AAACAAAACA | CATAGATTCA | CATGTAAAAC |

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FIGURE 1C

2701 TTTATTTTAT ATAATGAAGT ATTCCACCTT TAAATTAAAC AATTTATTTT
2751 ATTTTAGCAA TTCCGCTGAT AGAAAACAAG AGTGGAAAAA GAAACTTTTA
2801 TAAATTAAGT ATACGTATGT ACAAATGTGT TATGTGCCAT ATGTAGCAAT
2851 TTTTACAGT ATTTCCAAAA TGGGGAAAGA TATCAATGGT GCCTTTATGT
2901 TATGTTATGT TGAGAGCAAG TTTTGTACAG CTACAATGAT TGCTGTCCCG
2951 TAGTATTTTG CAAAACCTTC TAGCCCTCAG TTGTTCTGGC TTTTTTGTGC
3001 ATTGCATTAT AATGACTGGA TGTATGATTT GCAAGAATTG CAGAAGTCCC
3051 CATTTGCTTG TTGTGGAATC CCCAGATCAA AAAGCCCTGT TATGGCACTC
3101 ACACCCTATC CACTTCACCA GGAAAAAAAA AAAATCAAAA AAAAAAAAAA
3151 AAAAAAAGA AAAGAAAGAG AAAAAAGAAA AGAAAAAGAA AAAAAAGCT
3201 GAAAAAATAA AA

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FIGURE 2

1 GCCCYCHFCR CRCCYRFCFC SFYRMTIVFL A*ADTSLRCS R*ESCLSLWA
 51 IGSLNPVNLF AARGCLSPRP PSPCFVLFRL LSGGRSFPQS EELELLERRI
 101 RNYKSGQETR AQ*LQSCPWL RQGSVSGRGL GQGAGGLLFP VRSSSPSSDD
 151 VAVSDLSLTP AL*FLLSAVT VTPSLSVCVS Q*WTVIELRP FGGELCHS*C
 201 LNMSEVGTET FPSPSAQLSP DASLGGLPAE ENMPGPHRED SRVPGVAGLA
 251 STCCVCLEAE RLKGCLNSEK ICIAPILACL LSLCLCIAGL KWVFVDKIFE
 301 YDSPTHLDPG RIGQDPRSTV DPTALSAWVP SEVYASPFPI PSLESKA EVT
 351 VQTDSSLVPS RPFLQPSLYN RILDVGLWSS ATPSLSPSSL EPTTASQAQA
 401 TETNLQTAPK LSTSTSTTGT SHLTCKDIKQ KAFCVNGGEC YMVKDLPNPP
 451 RYLCRCPNEF TGDRCONYVM ASFYKHLGIE FMEAEELYQK RVLITITGICI
 501 ALLVVGIMCV VAYCKTKKQR KKLHDRLRQS LRSENNVMN MANGPHHPNP
 551 PPDNVQLVNQ YVSKNIISSE RVVERETETS FSTSHYTSTT HHSMTVTQTP
 601 SHSWSNGHTE SILSESHSVL VSSSVENSRH TSPTGPRGRL NGIGGPREGN
 651 SFLRHARETP DSYRDSPHSE RYVSAMTTTPA RMSPVDFHTP TSPKSPPEM
 701 SPPVSSLTIS IPSVAVSPFM DEERPLLLVT PPRLREKYDN HLQQFNSFHN
 751 NPTHESNSLP PSPLRIVEDE EYETTQEYEP AQEPPKKLTN SRRVKRTKPN
 801 GHISSRVEVD SDTSSQSTSS ESETEDERIG EDTPFLSIQN PMATSLEPAA
 851 AYRLAENRTN PANRFSTPEE LQARLSSVIA NQDPIAV*DI NKTHRFTCKT
 901 LFYIMKYSTF KLNNLFYFSN SADRKQEWKK KLL*IKYTYV QMCYVPYVAI
 951 FYSISKMGKD INGAFMLCYV ESKFCTATMI AVP*YFAKPS SPQLFWLFCA
 1001 LHYNDWMYDL QELQKSPFAC CGIPRSKSPV MALTPYPLHQ EKKKIKKKKK
 1051 KKRKEREKRK EKEKKS*KNK

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FIGURE 3

1 CGGCCTGTAA GATGCTGTAT CATTGTTG GGGGGCCTC TGCCTGGTAA
 51 TGGACCGTGA GAGCGGCCAG GCCTTCTTCT GGAGGTGAGC CGATGGAGAT
 101 TTATTCCCCA GACATGTCTG AGGTCGCCGC CGAGAGGTCC TCCAGCCCCT
 151 CCACTCAGCT GAGTGCAGAC CCATCTCTTG ATGGGCTTCC GGCAGCAGAA
 201 GACATGCCAG AGCCCCAGAC TGAAGATGGG AGAACCCTG GACTCGTGGG
 251 CCTGGCCGTG CCCTGCTGTG CGTGCCTAGA AGCTGAGCGC CTGAGAGGTT
 301 GCCTCAACTC AGAGAAAATC TGCATTGTCC CCATCCTGGC TTGCCTGGTC
 351 AGCCTCTGCC TCTGCATCGC CGGCCTCAAG TGGGTATTTG TGGACAAGAT
 401 CTTTGAATAT GACTCTCCTA CTCACCTTGA CCCTGGGGGG TTAGGCCAGG
 451 ACCCTATTAT TTCTCTGGAC GCAACTGCTG CCTCAGCTGT GTGGGTGTCTG
 501 TCTGAGGCAT AACTTCACC TGTCTCTAGG GCTCAATCTG AAAGTGAGGT
 551 TCAAGTTACA GTGCAAGGTG ACAAGGCTGT TGTCTCCTTT GAACCATCAG
 601 CGGCACCGAC ACCGAAGAAT CGTATTTTTG CCTTTTCTTT CTTGCCGTCC
 651 ACTGCGCCAT CCTTCCCTTC ACCCACCCGG AACCTGAGG TGAGAACGCC
 701 CAAGTCAGCA ACTCAGCCAC AAACAACAGA AACTAATCTC CAAACTGCTC
 751 CTAAACTTTC TACATCTACA TCCACCACTG GGACAAGCCA TCTTGTAATA
 801 TGTGCGGAGA AGSAGAAAAC TTTCTGTGTG AATGGAGGGG AGTGCTTCAT
 851 GGTGAAAGAC CTTTCAAACC CCTCGAGATA CTTGTGCAAA GGCGGAGGAG
 901 CTGTACCAGA AGAGAGTGCT GACCATAACC GGCATCTGCA TCGCCCTCCT
 951 TGTGGTCGGC ATCATGTGTG TGGTGGCCTA CTGCAAAACC AAGAAACAGC
 1001 GGAAAAAGCT GCATGACCGT CTTCCGGCAGA GCCTTCGGTC TGAACGAAAC
 1051 AATACGATGA ACATTGCCAA TGGGCCTCAC CATCCTAACC CACCCCCCGA
 1101 GAATGTCCAG CTGGTGAATC AATACGTATC TAAAAACGTC ATCTCCAGTG
 1151 AGCATATTGT TGAGAGAGAA GCAGAGACAT CCTTTTCCAC CAGTCACTAT
 1201 ACTTCCACAG CCCATCACTC CACTACTGTC ACCCAGACTC CTAGCCACAG
 1251 CTGGAGCAAC GGACACACTG AAAGCATCCT TTCCGAAAGC CACTCTGTAA
 1301 TCGTGATGTC ATCCGTAGAA AACAGTAGGC ACAGCAGCCC AACTGGGGCC
 1351 G

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FIGURE 4

1 ACKMLYHLVG GASAW*WTVR AARPSSGGEP MEIYSPDMSE VAAERSSSPS
51 TQLSADPSLD GLPAAEDMPE PQTEDGRTPG LVGLAVPCCA CLEAERLRGC
101 LNSEKICIVP ILACLVSLCL CIAGLKWV FV DKIFEYDSPT HLDPGGLGQD
151 PIISLDTAA SAVVVSSEAY TSPVSRAQSE SEVQVTVQGD KAVVSFEPSA
201 APTPKNRIFA FSFLPSTAPS FPSPTRNPEV RTPKSATQPQ TTETNLQTAP
251 KLSTSTSTTG TSHLVKCAEK EKTFCVNGGE CFMVKDLSNP SRYLCKGGGA
301 VPEESADHNR HLRPPCGRH HVCGGLLQNO ETAEKAA*PS SAEPSV*TKQ
351 YDEHCQWASP S*PTPRECPA GESIRI*KRH LQ*AYC*ERS RDILFHQSLY
401 FHSPSLHYCH PDS*PQLEQR TH*KHPFRKP LCNRDVIRRK Q*AQQPNWG

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FIGURE 5A

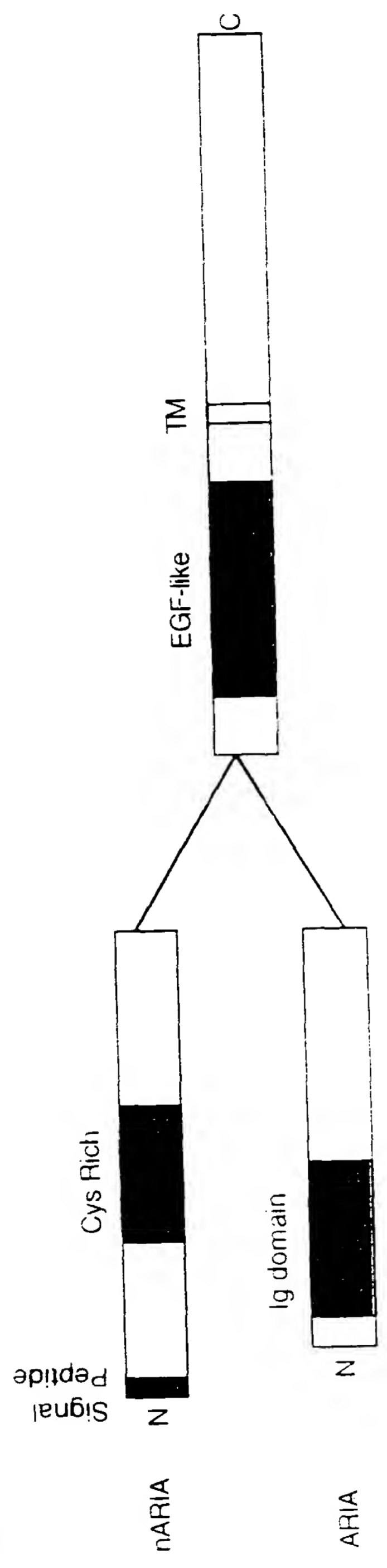


FIGURE 5B

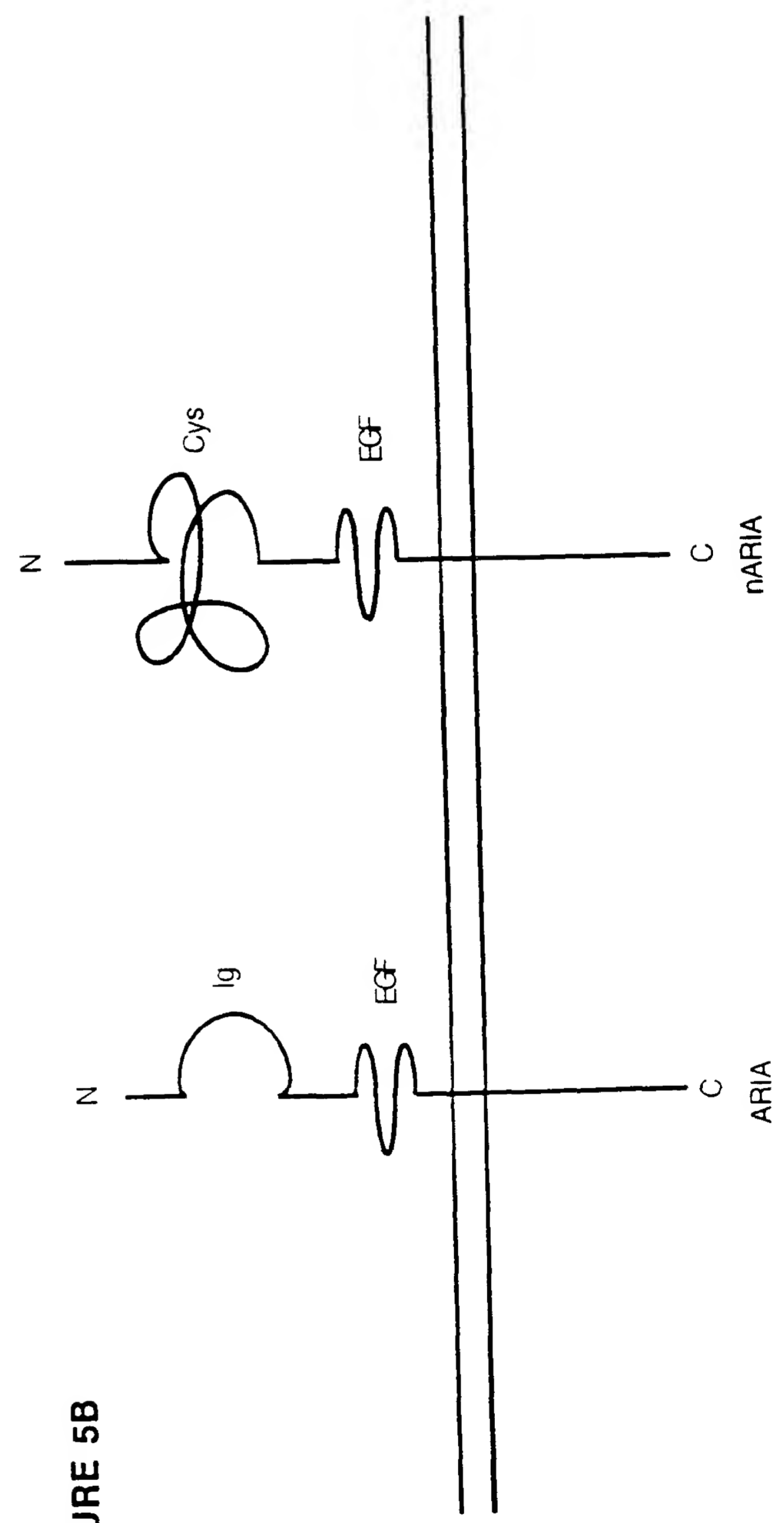
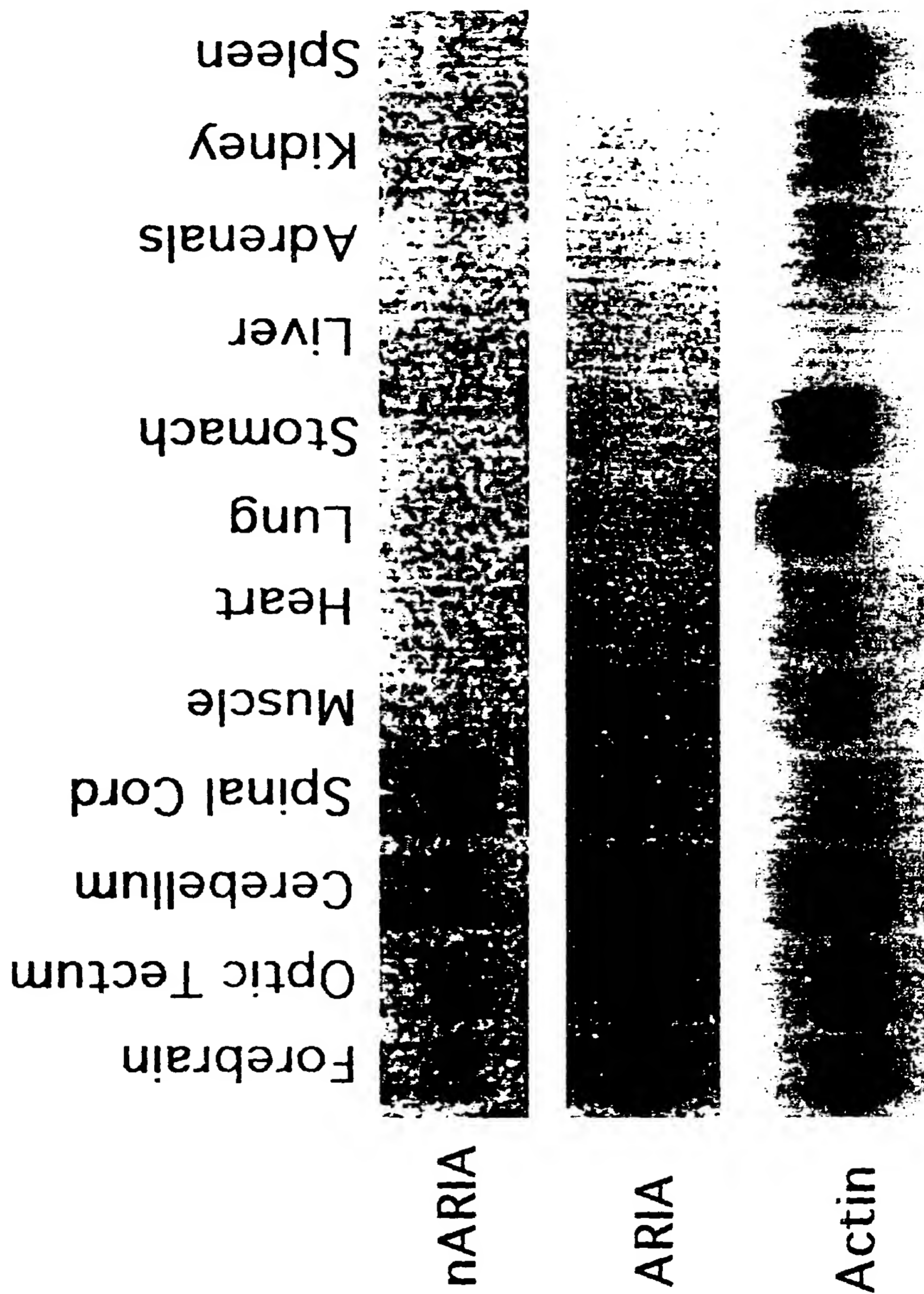


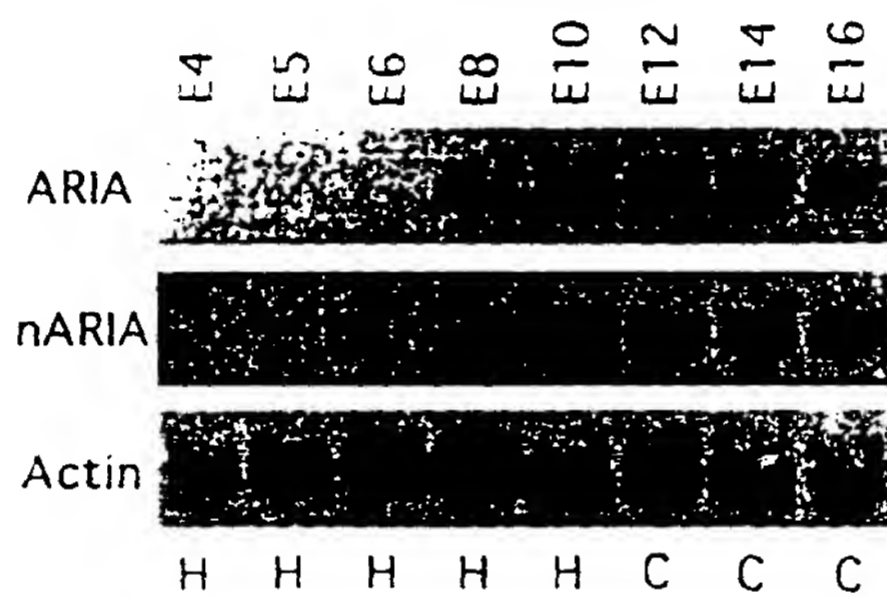
FIGURE 6



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FIGURE 7

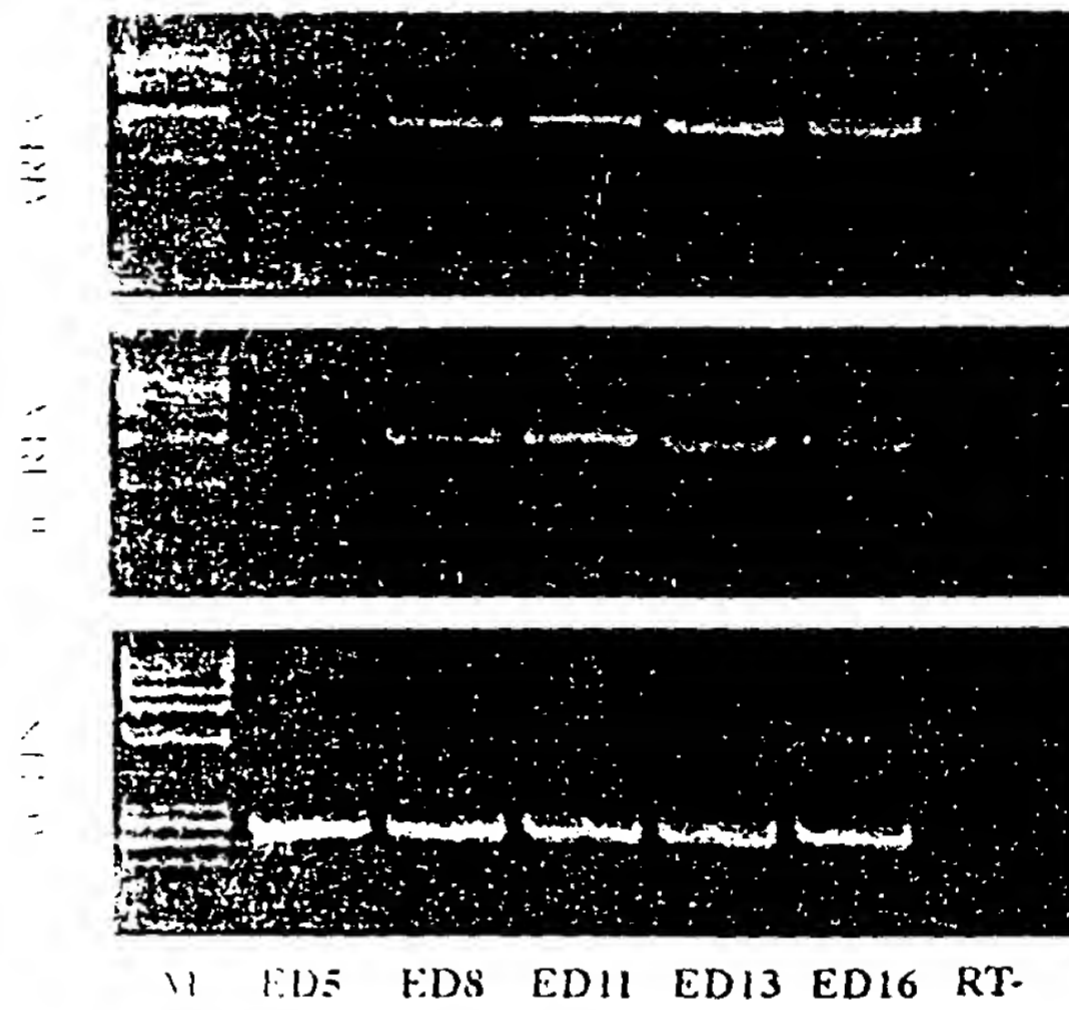
Developmental Northern of ARIA
and nARIA in the chick hindbrain
and cerebellum



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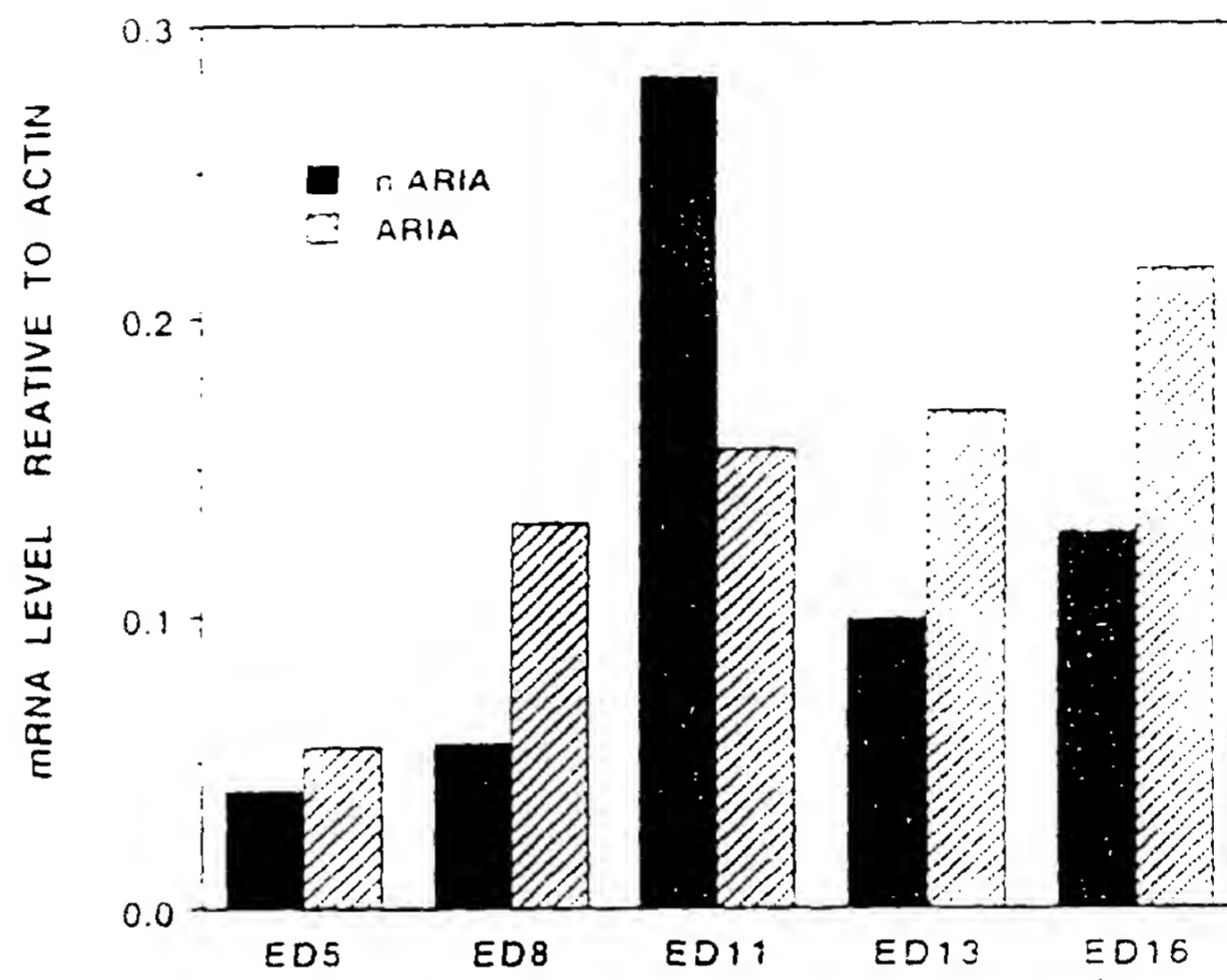
FIGURE 8A



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FIGURE 8B



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664750-9992160

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FIGURE 9A

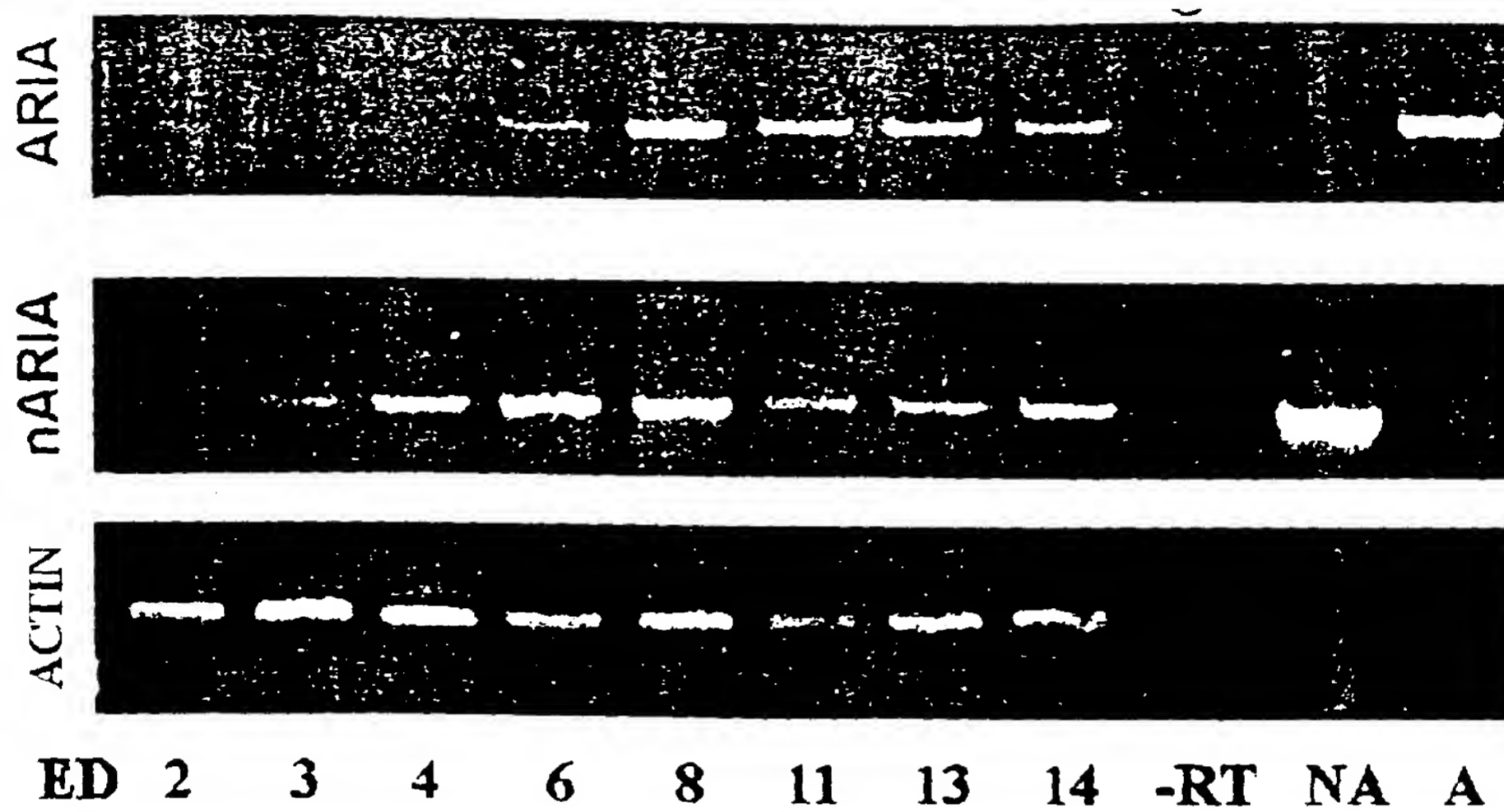
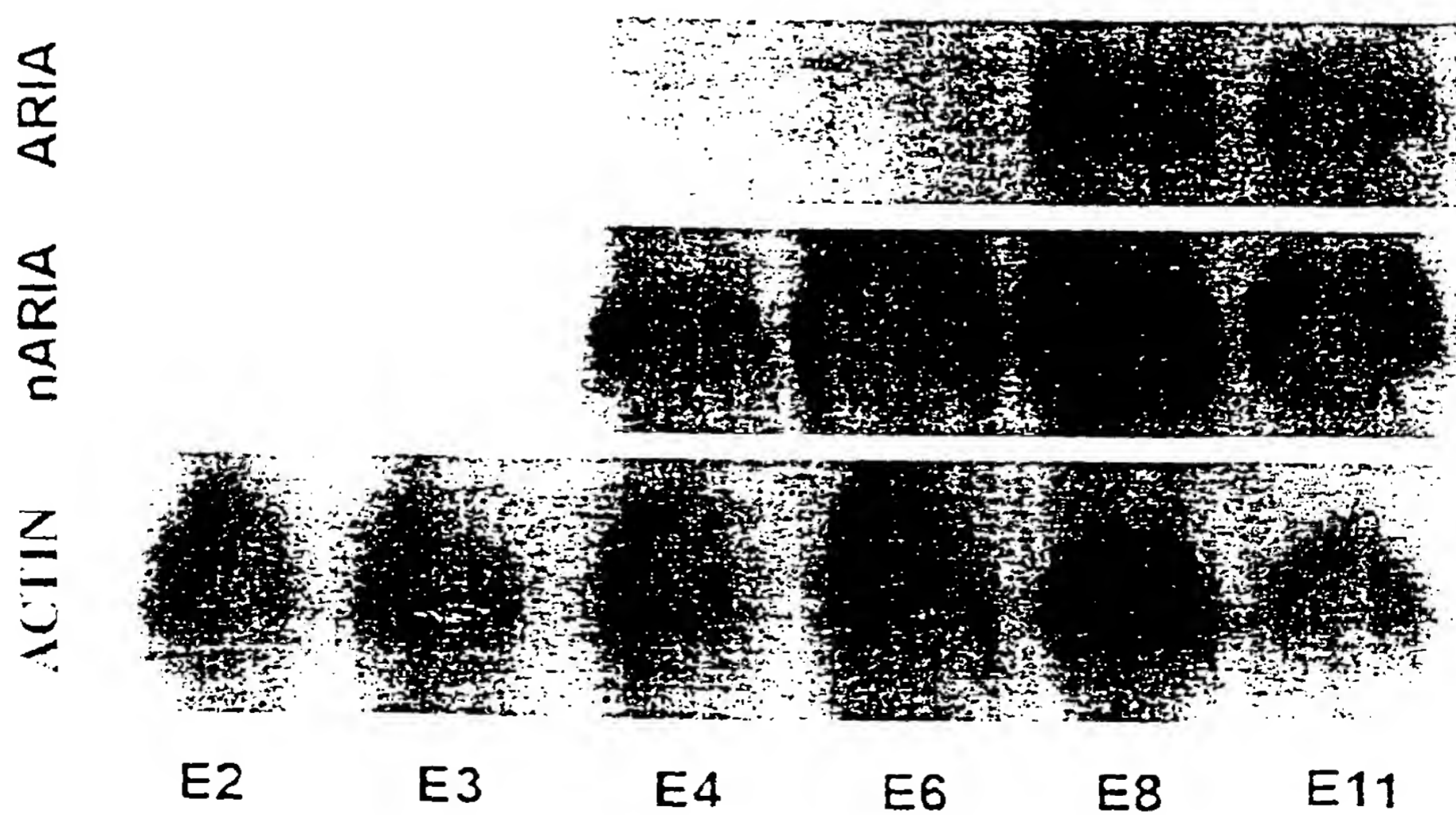


FIGURE 9B



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FIGURE 9C

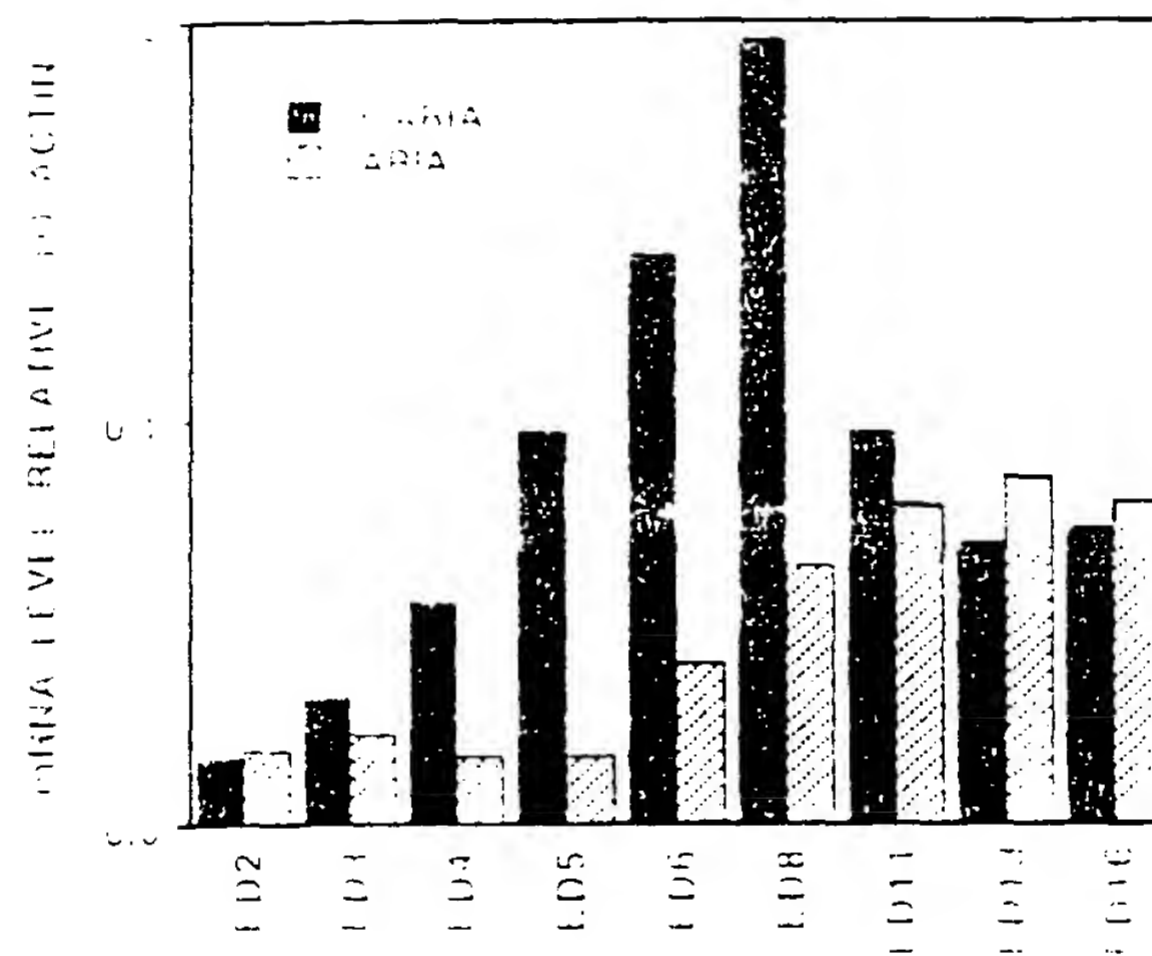
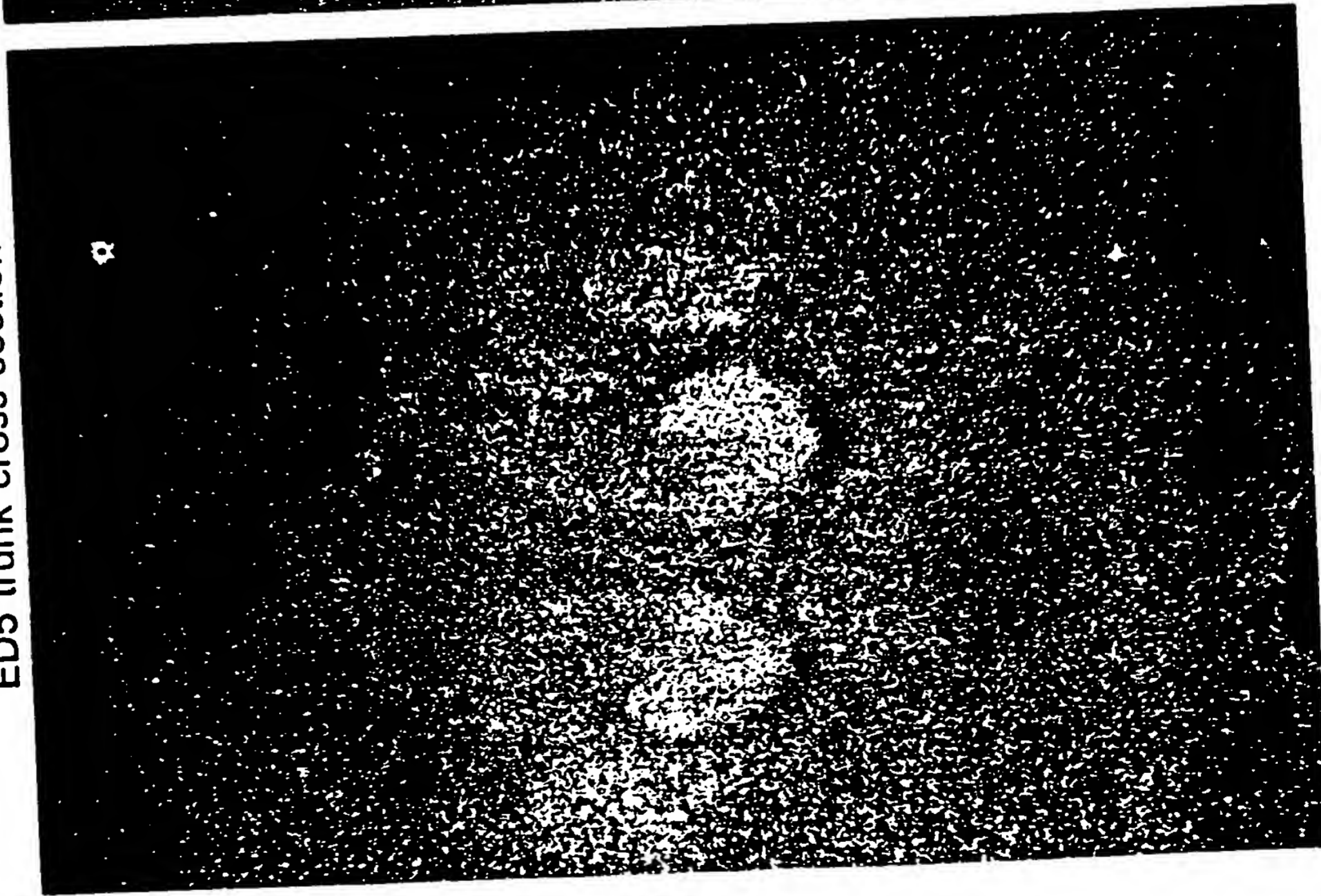
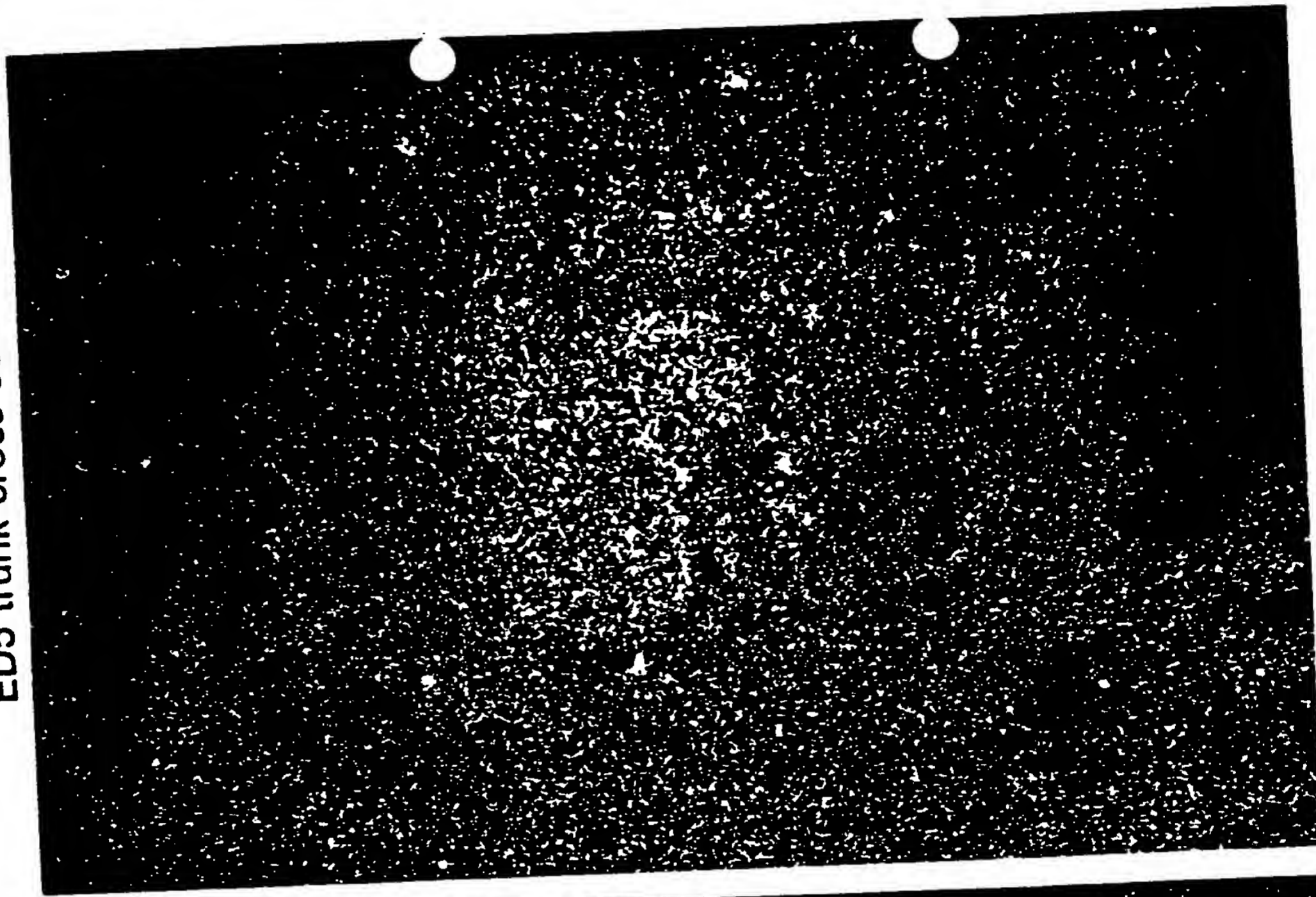


FIGURE 10A
ED5 trunk cross-section



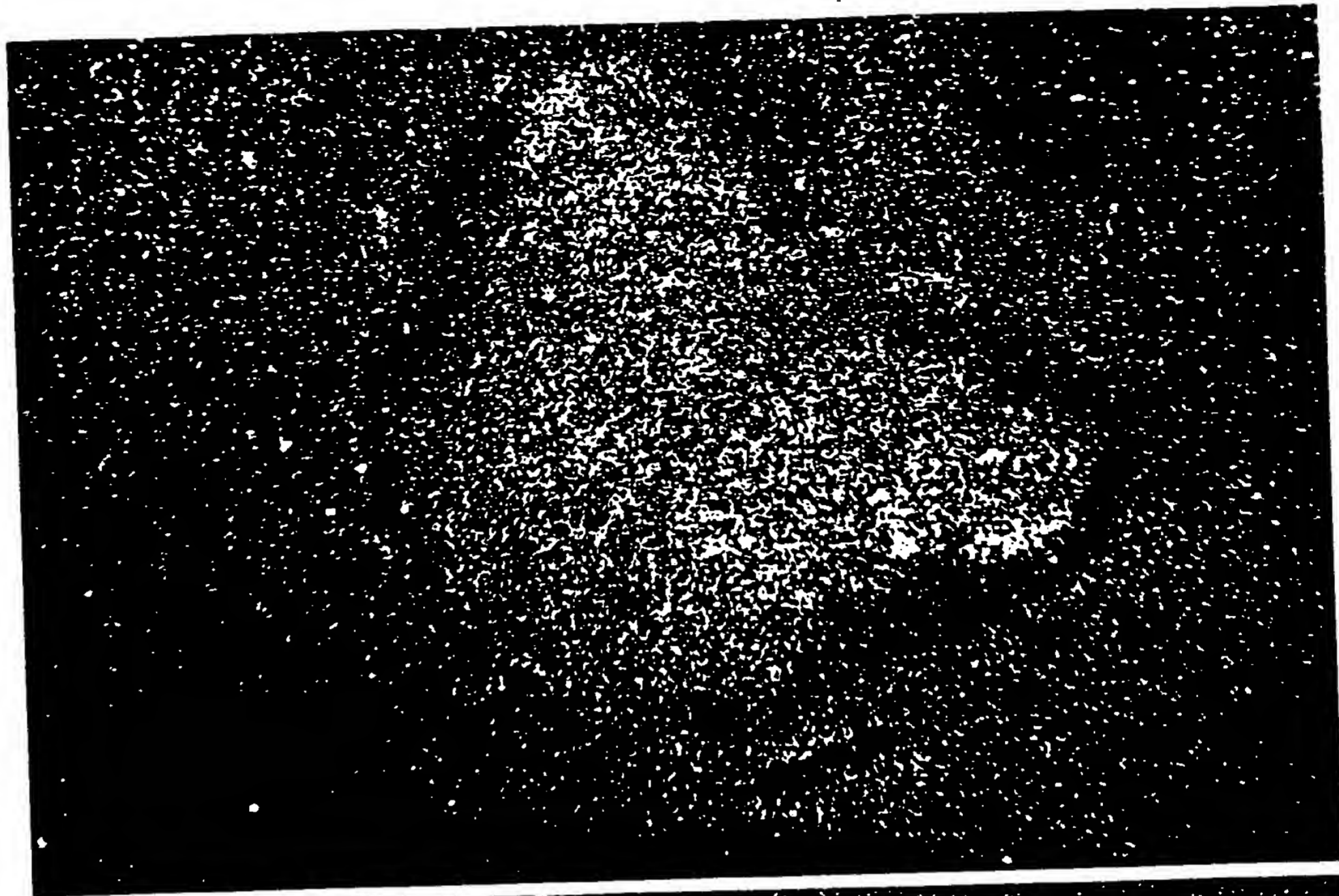
nARIA specific probe

FIGURE 10B
ED5 trunk cross-section



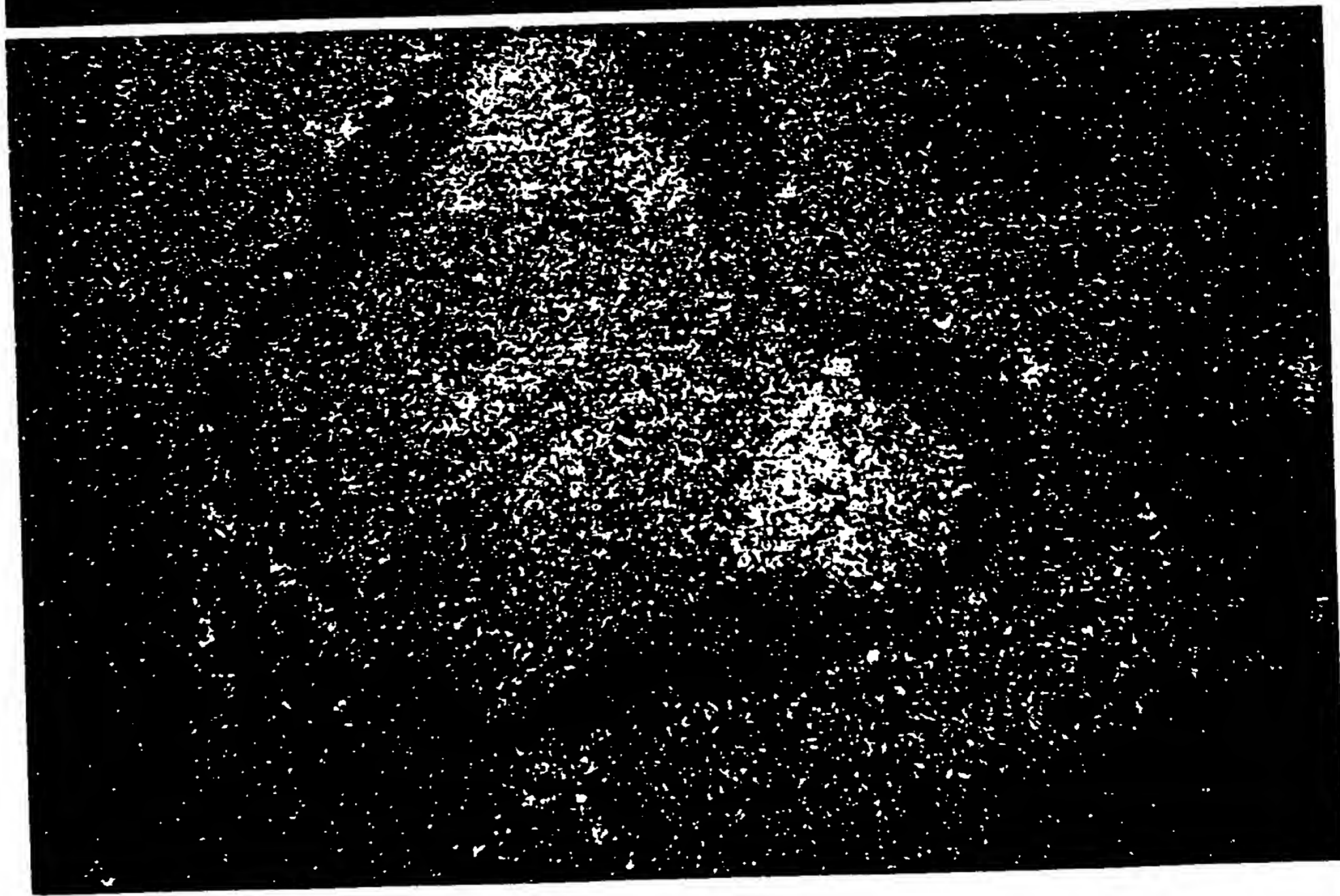
ARIA specific probe

FIGURE 10D
ARIA specific probe



ED7 trunk cross-section

FIGURE 10C
nARIA specific probe



ED7 trunk cross-section

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FIGURE 11A

FIGURE 11B

A. MCF-7

B. LSG

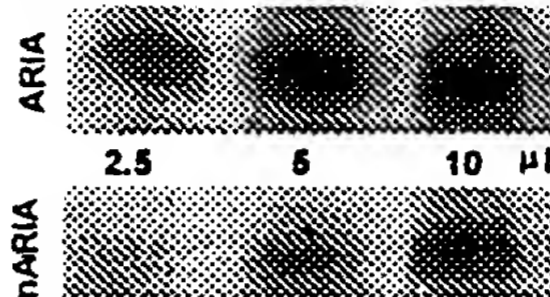
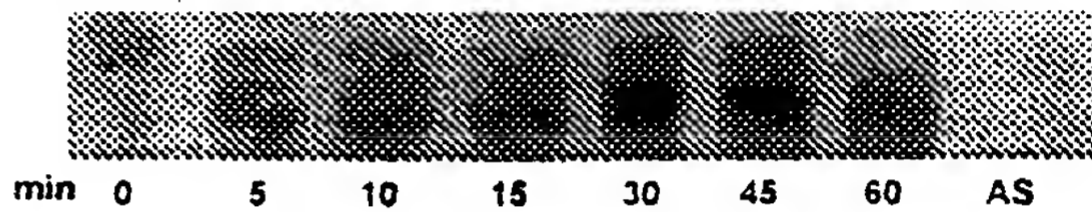
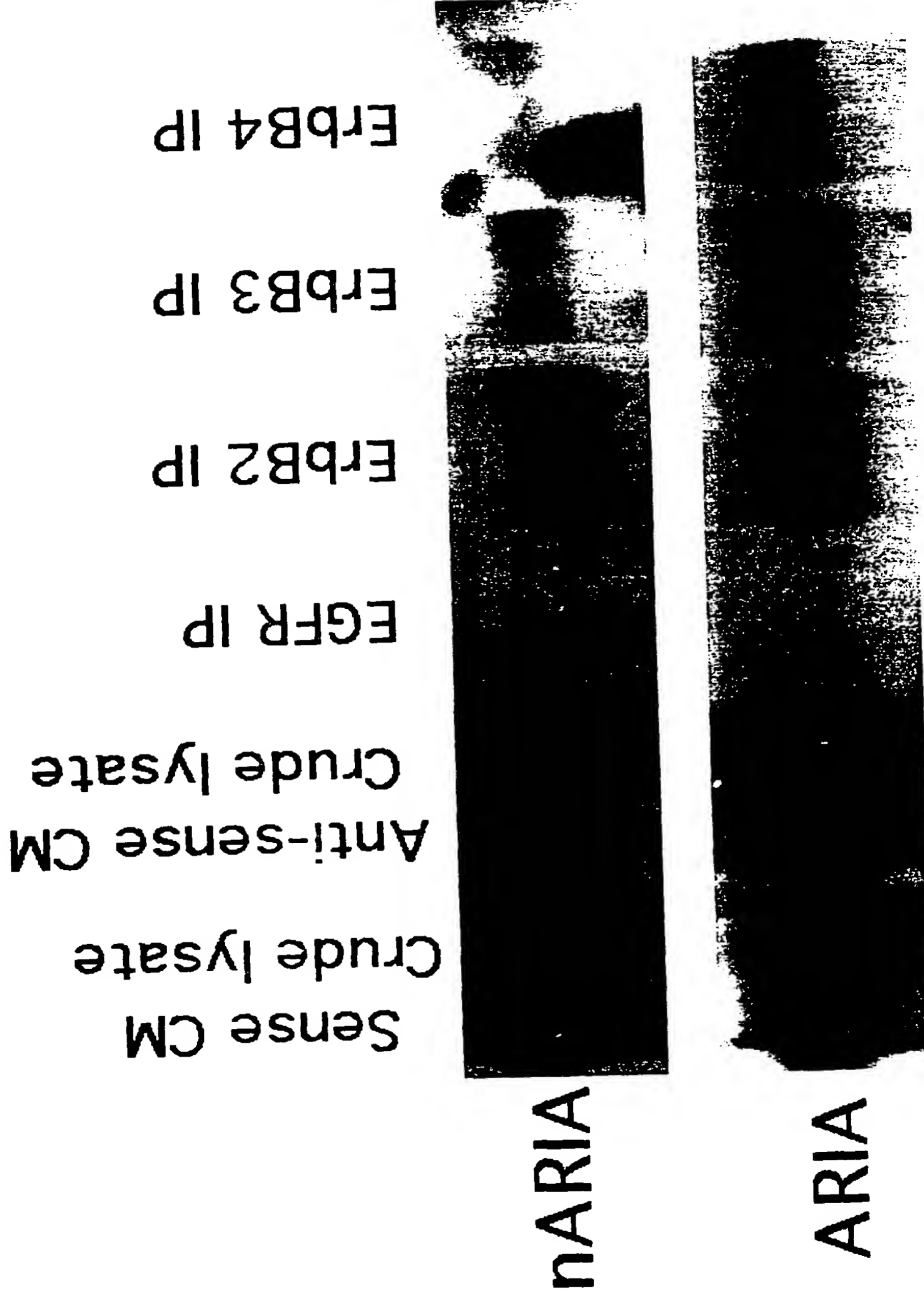


FIGURE 11C C. TIME COURSE



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FIGURE 12



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FIGURE 13A

ED9 5% ufCEE ACh response

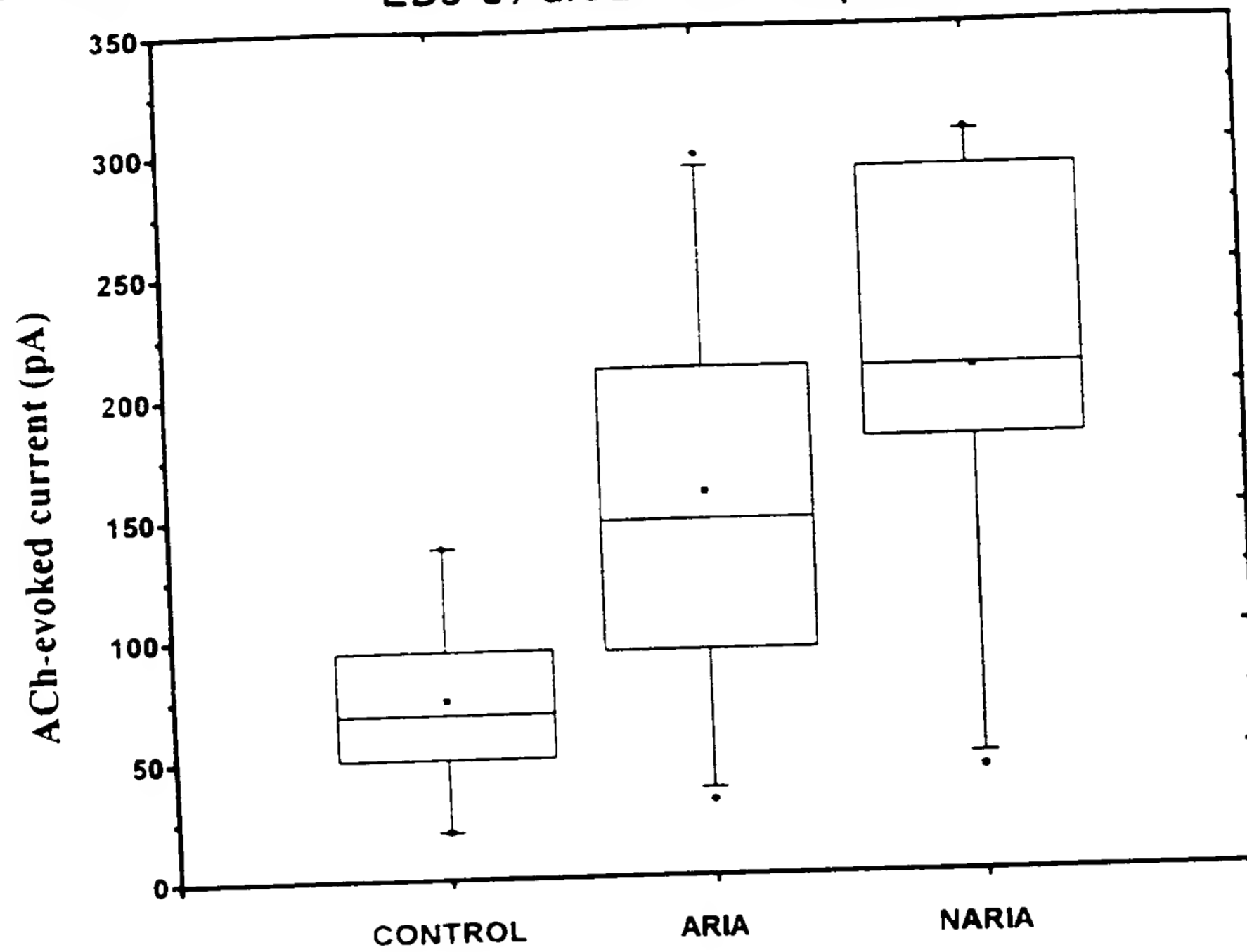


FIGURE 13B

ED9 2%ufCEE GABA response

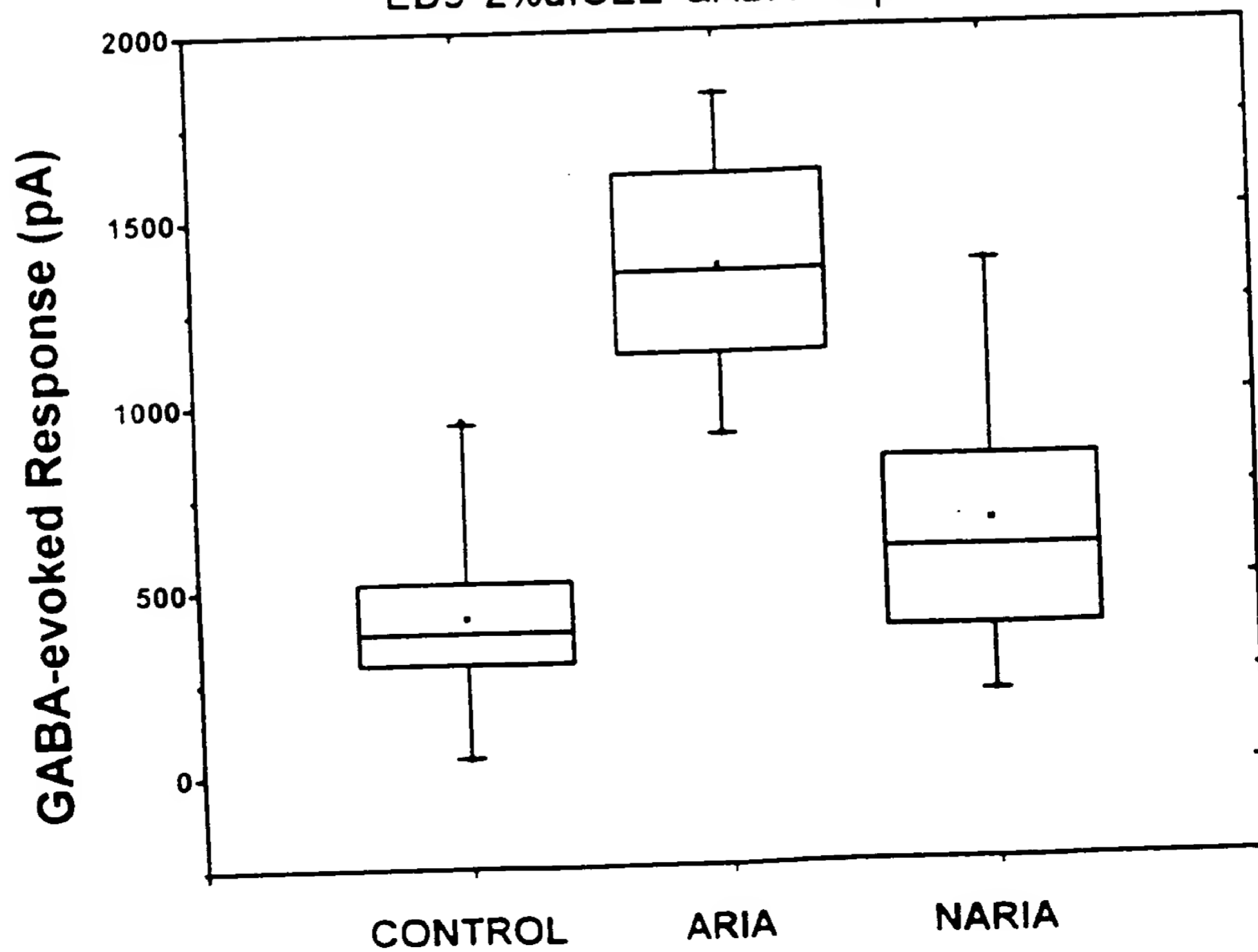


FIGURE 13C

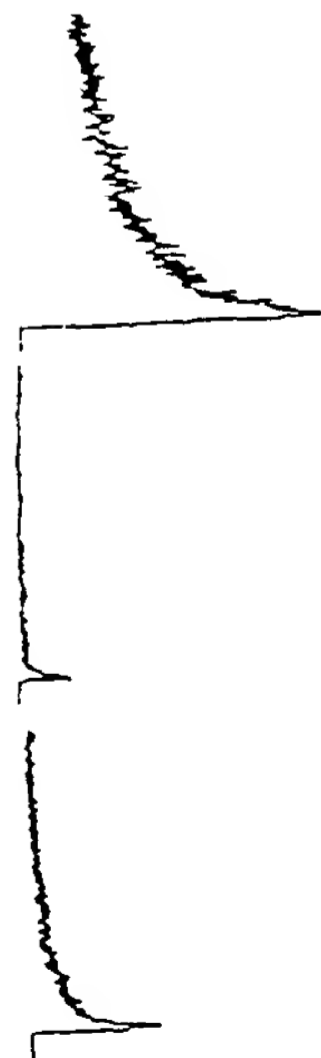
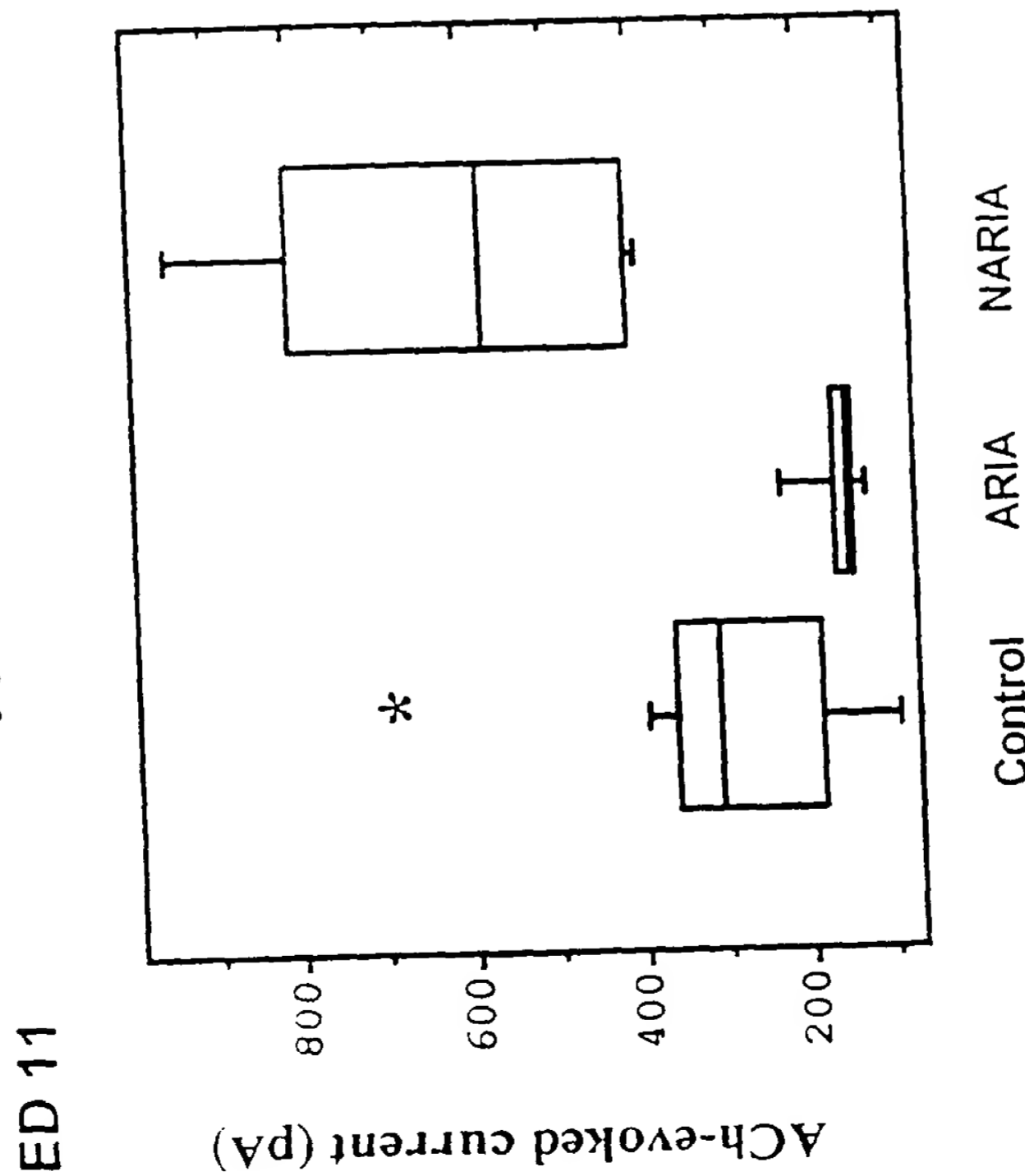


FIGURE 13D

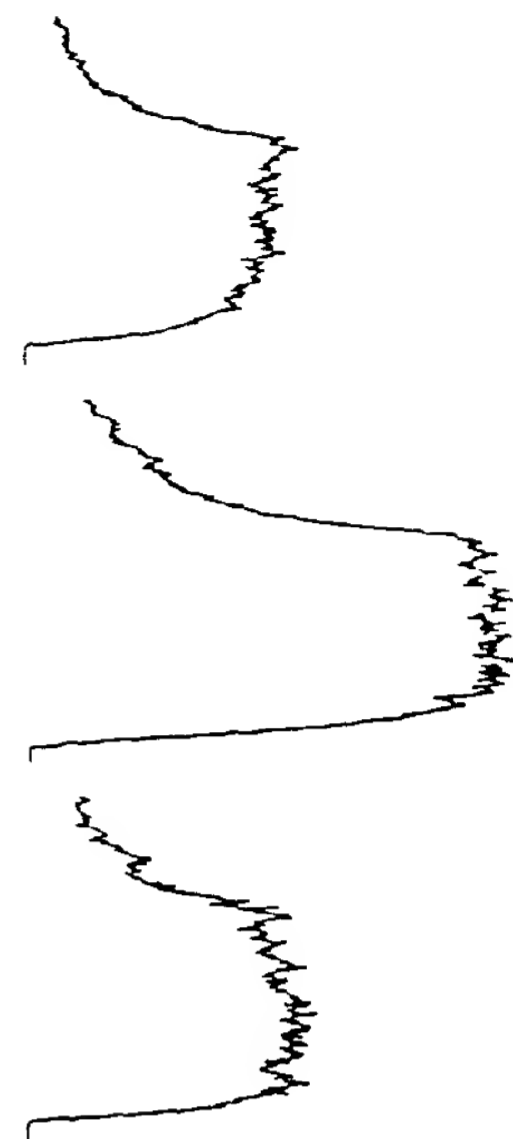
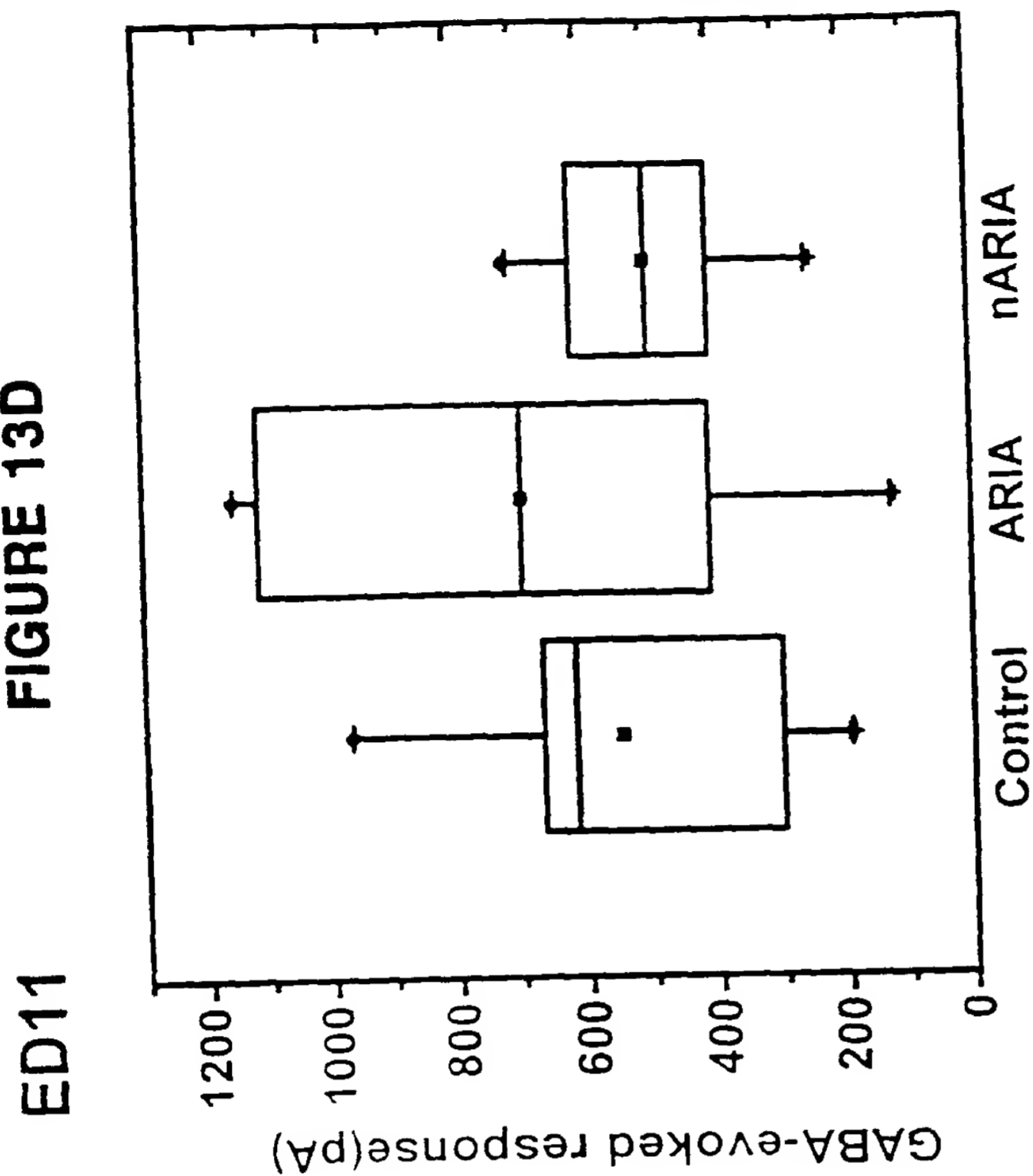


FIGURE 14B

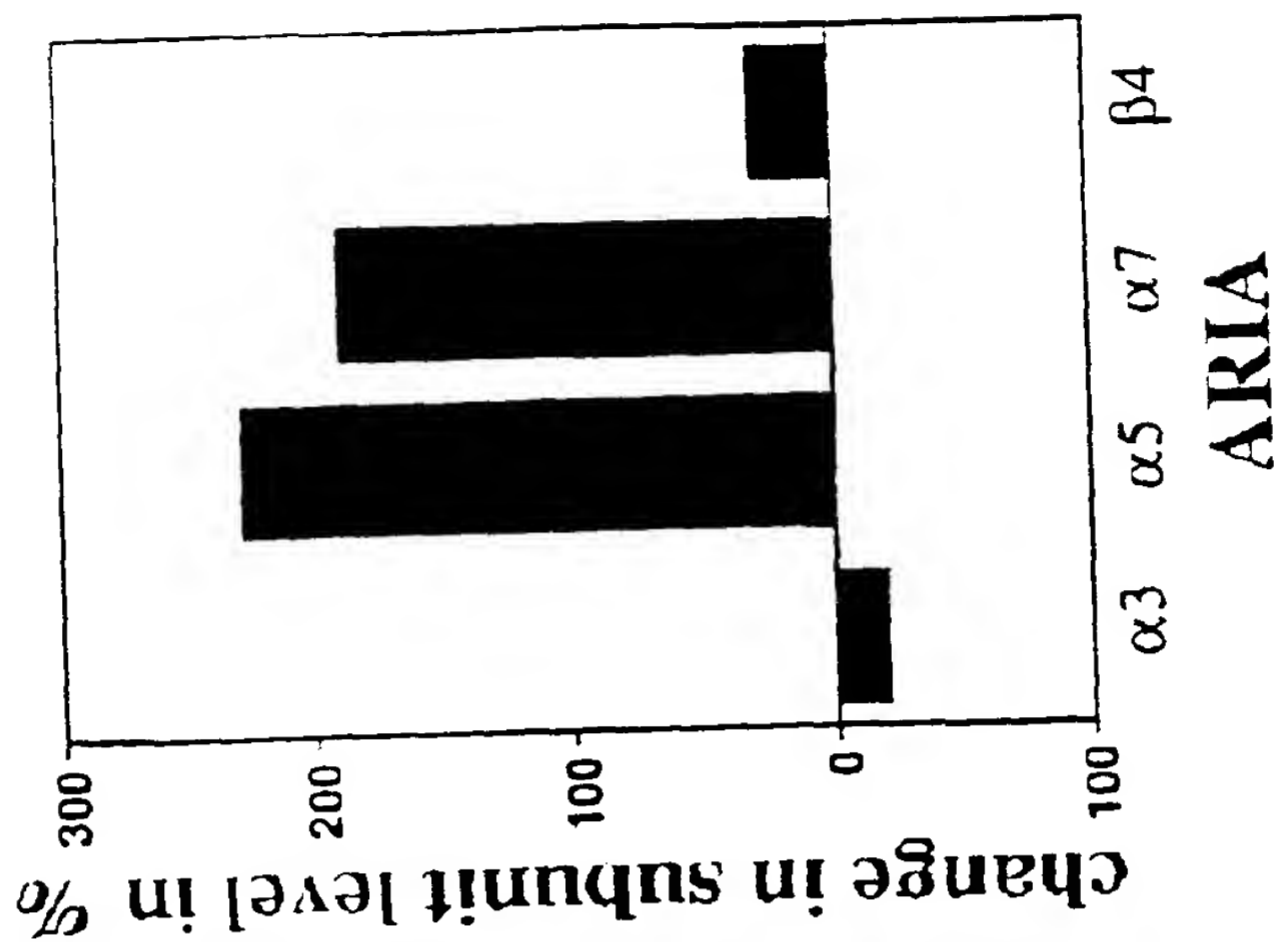


FIGURE 14A

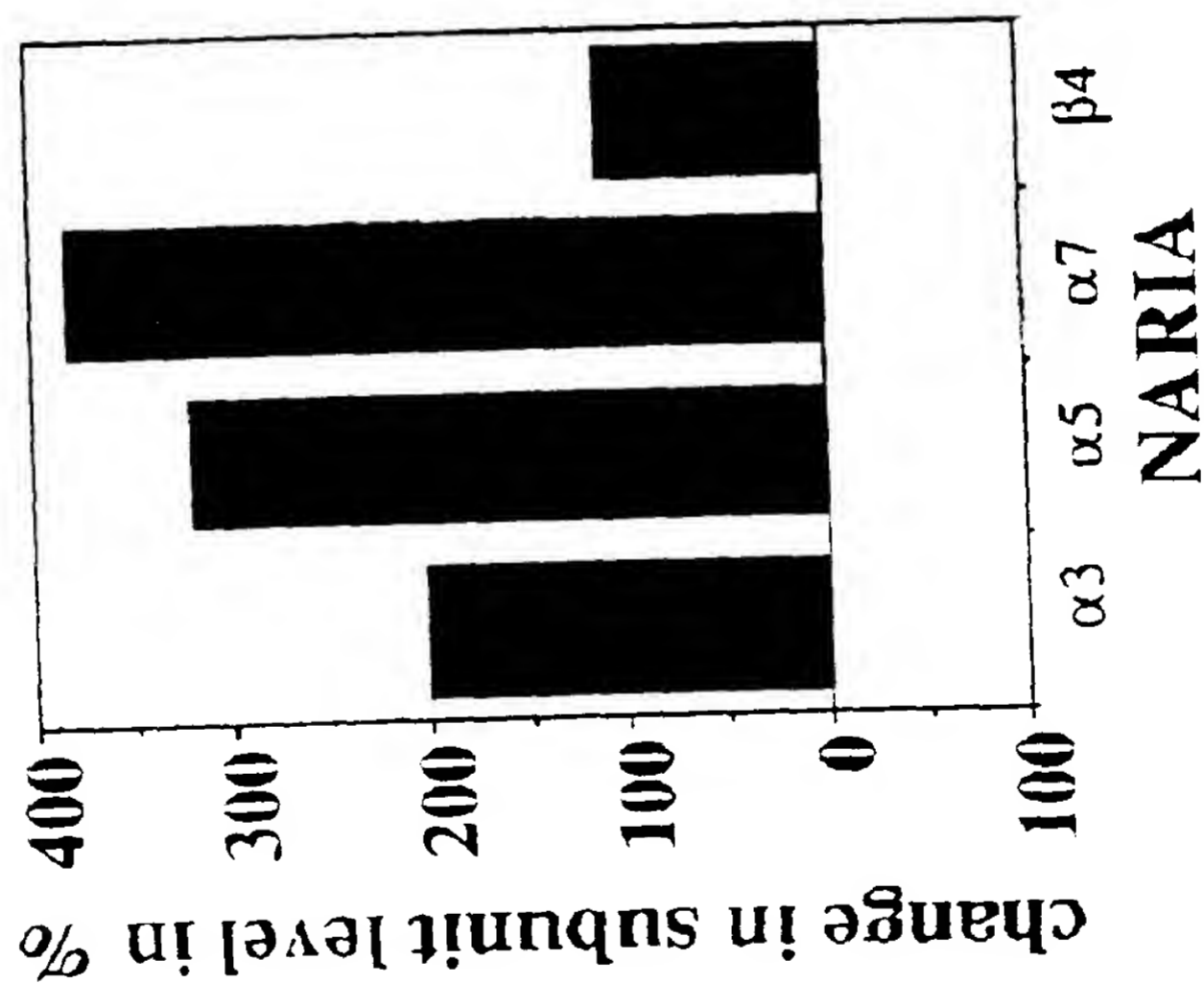
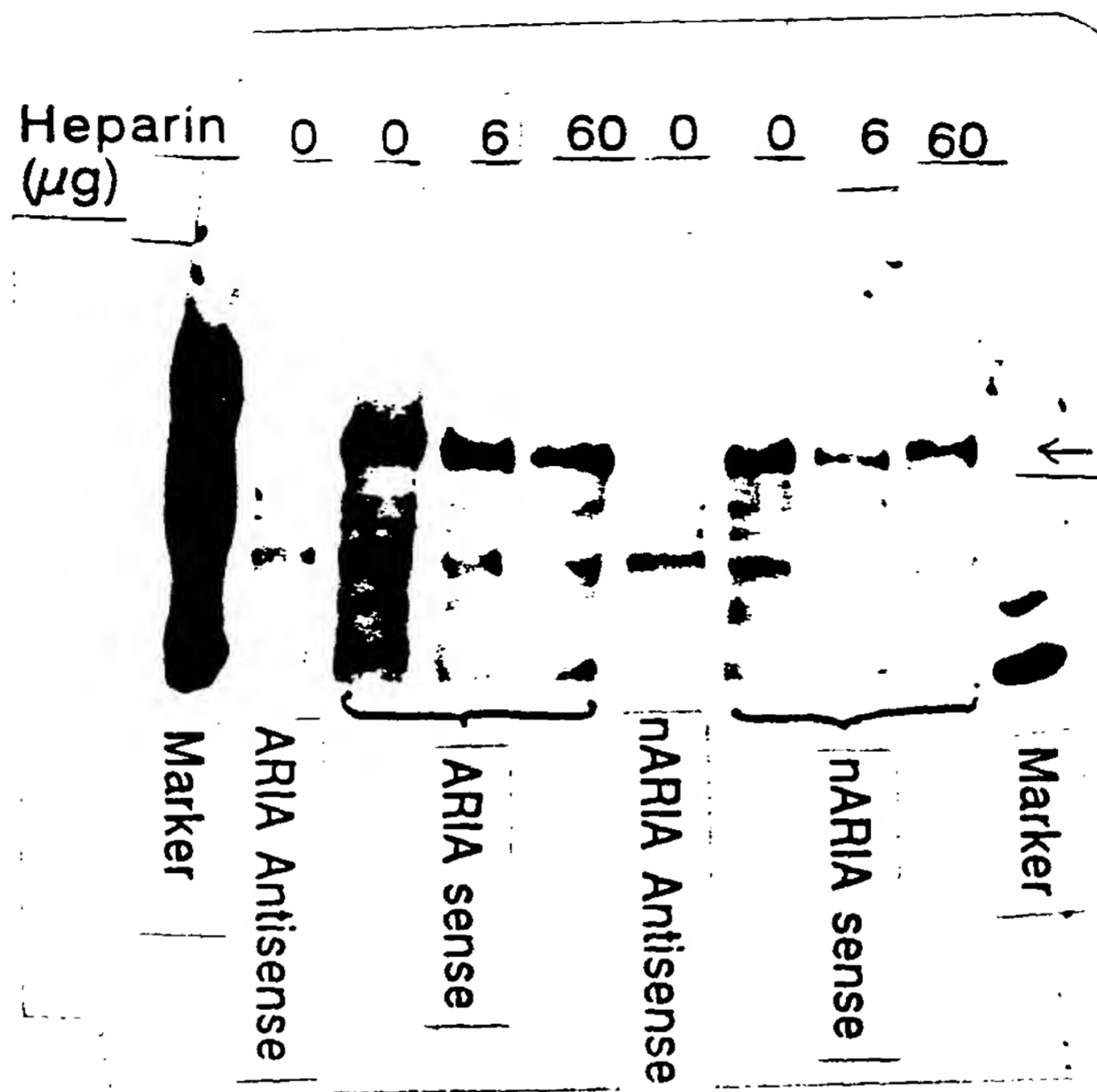
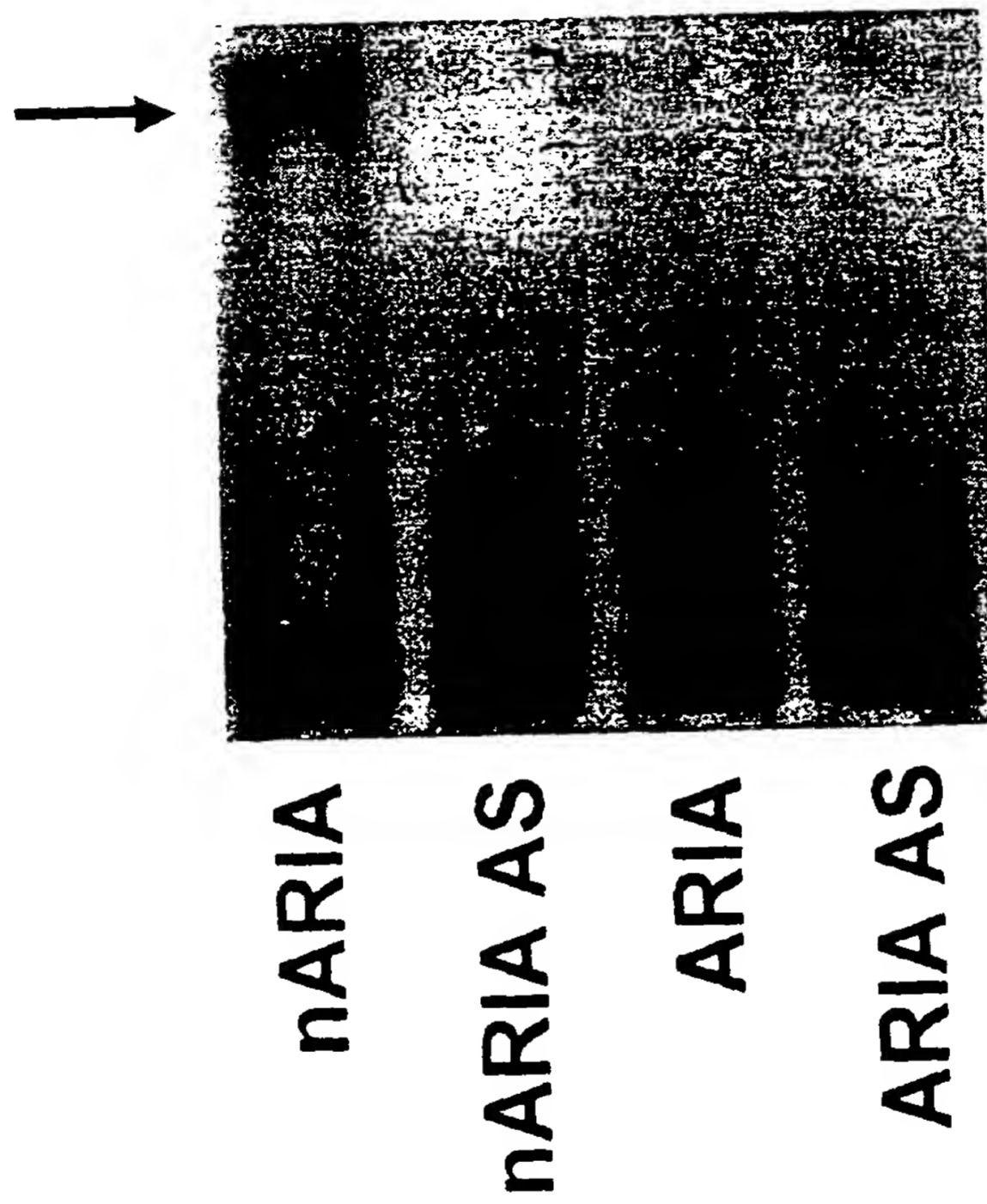


FIGURE 15



8% non-denaturing



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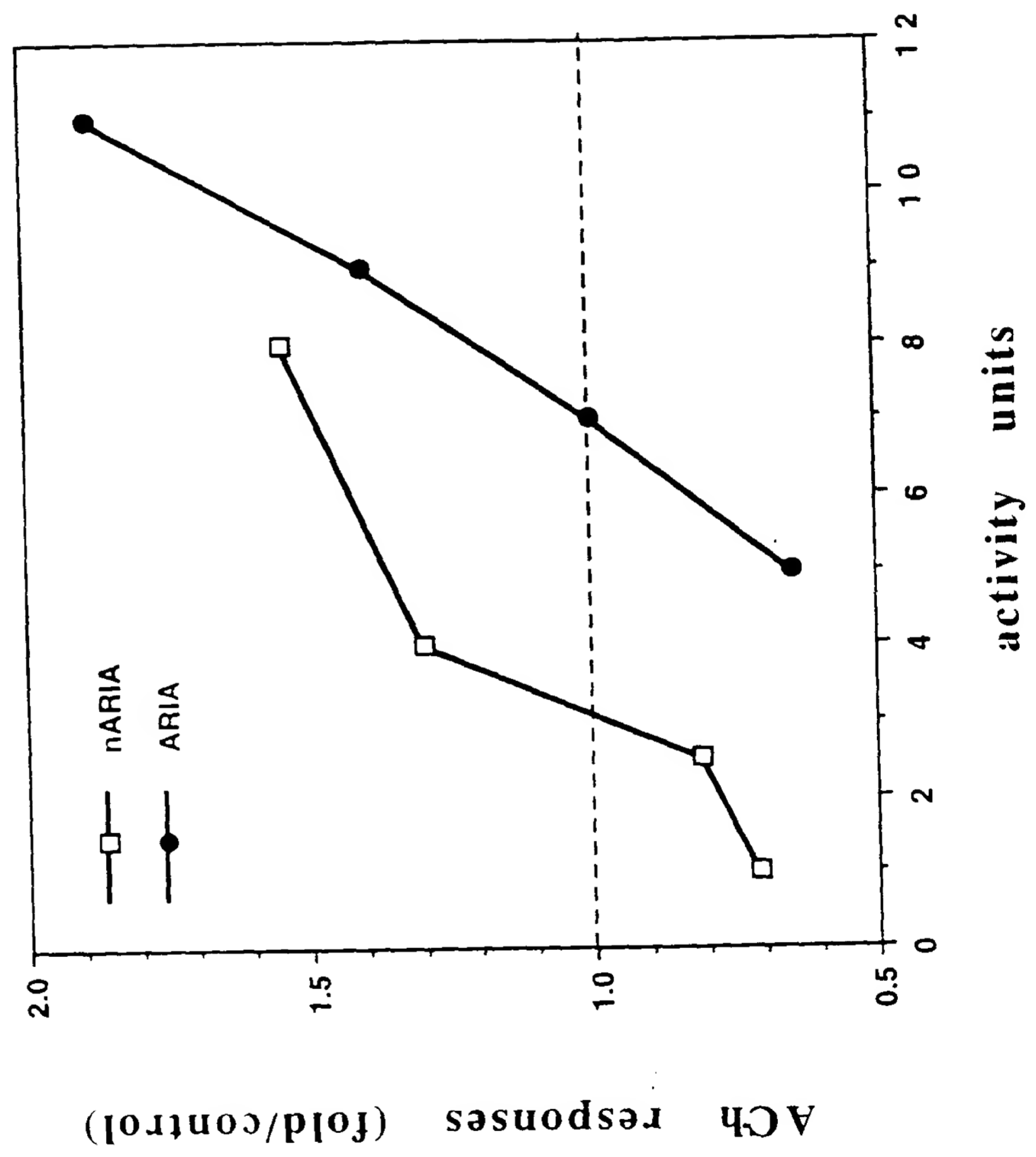
FIG. 17



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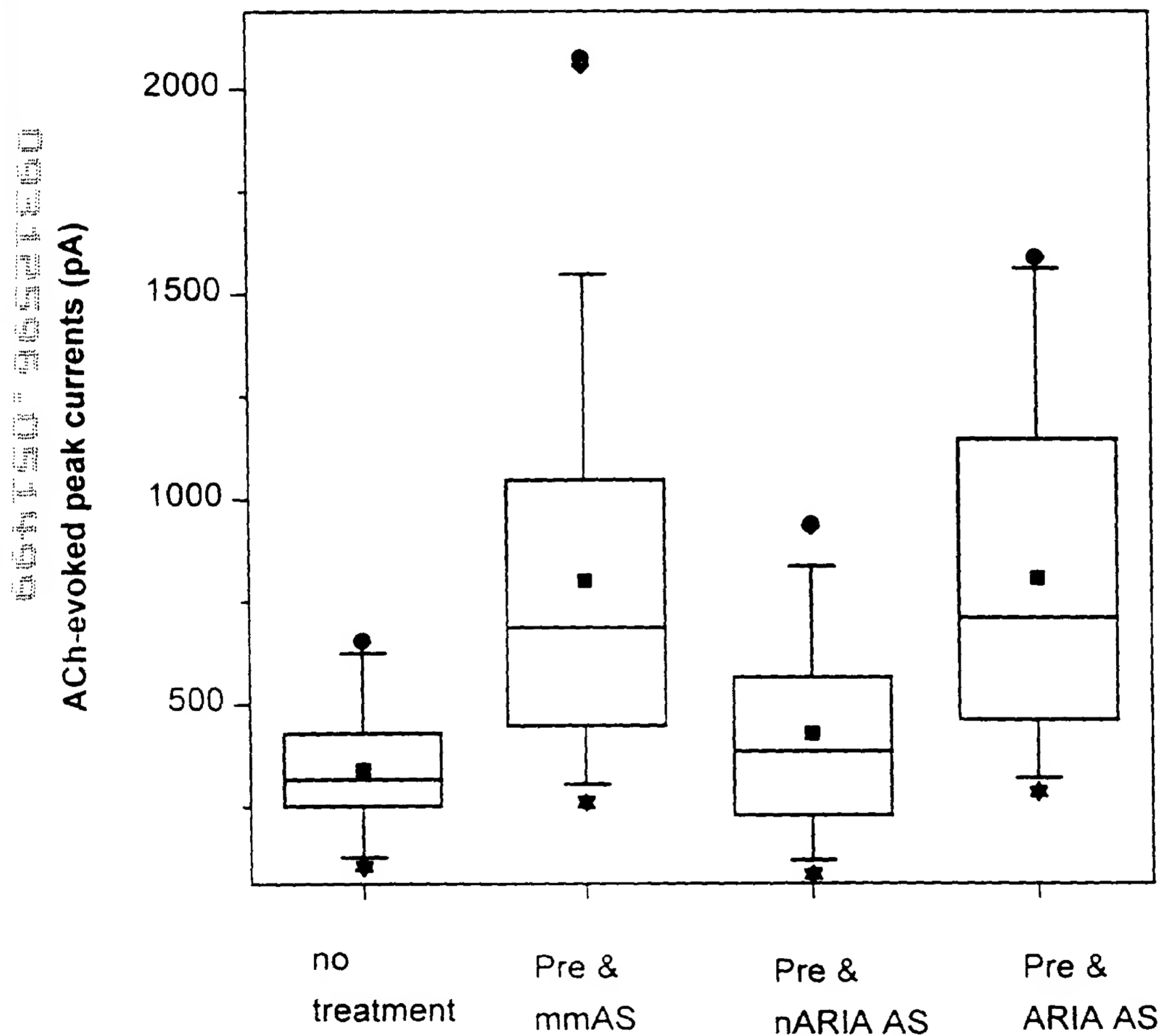
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FIG. 18



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FIG. 19



no treatment=sympathetic neurons alone

'Pre'=treatment of sympathetic neurons with presynaptic input-conditioned media+various oligos

mmAS=mismatch antisense control

nARIA AS=nARIA specific antisense oligonucleotides

ARIA AS=ARIA specific antisense oligonucleotides

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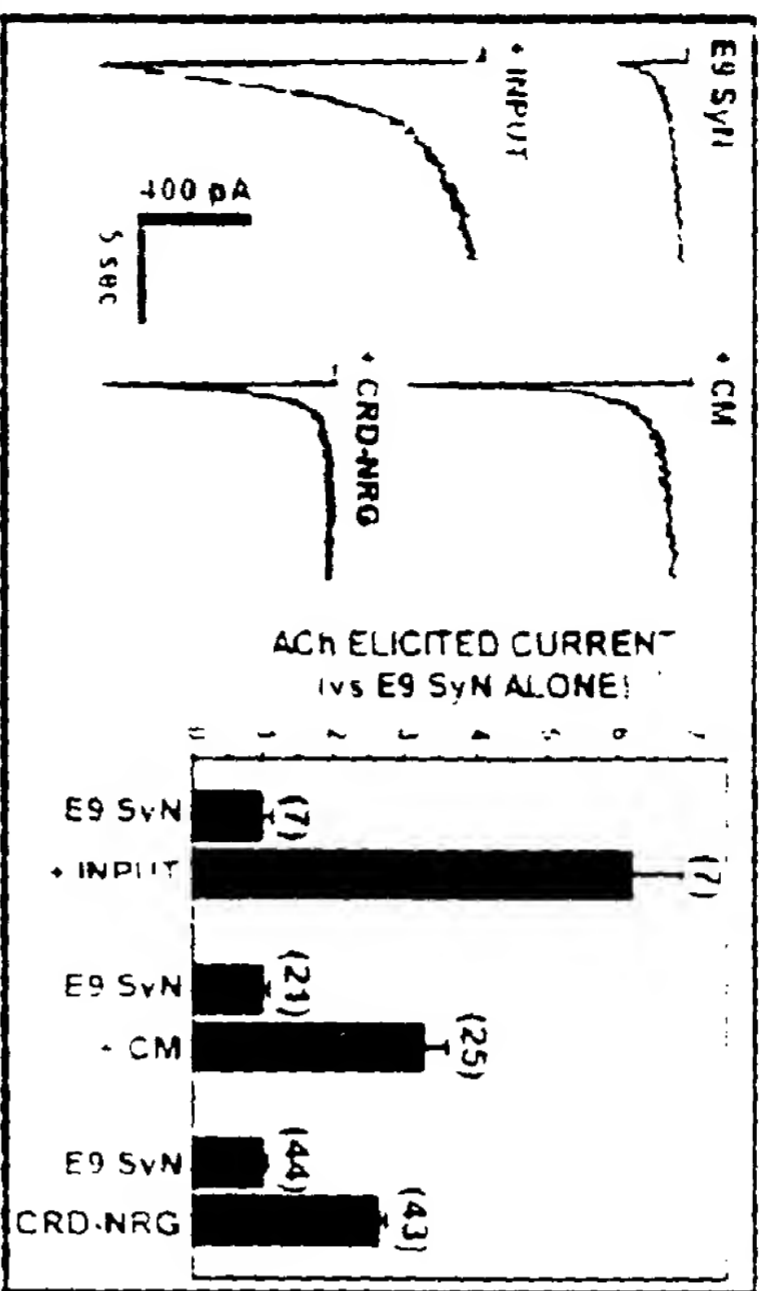
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| TABLE P1-1 | $\alpha 3$ | $\alpha 5$ | $\alpha 7$ | $\beta 4$ |
|---|------------------|------------------|------------------|------------------|
| SYMPs ALONE (set to $\equiv 1$) mRNA/SyMp (fg/100 fg std) | 1.2 ± 0.2 | 0.4 $\pm .01$ | 0.2 $\pm .05$ | 0.4 ± 0.1 |
| SyMps + INPUT | 4.9 | 6.6 | 18 | 1.6 |
| SyMps + TARGET | 0.6 | 6.3 | 3.0 | 4.6 |
| SYMPs+ INPUT+ TARGET | 2.7 | 10 | 23 | 10 |
| . <i>in vivo</i> DEVELOPMENT | 2.8 | 11 | 21 | 12 |

Anterograde (Input) and Retrograde (Target) co regulation of nAChR expression utilize distinct (~ additive) mechanisms.

nAChR mRNA were assayed from synaptically naive SyNs (E9 chick) *in vitro*. Conditions indicated & presented as fold change relative to E9 SyMps ($\equiv 1$). n= (from top):49, 51, 17, 31, 6 experiments of each condition. Single cell qPCR following electrophysiology, data corrected for amplification efficiency & actin standard (& Prog.: A2) ^RNase protection assay of E8 vs. E21, corrected for neuron number and actin standard. + heart target data: # kidney target data (see **Aim 2 Progress**)

A. TREATMENT with RECOMBINANT CRD-NRG MIMICS the INDUCTION of nAChRs BY INPUT & INPUT-DERIVED CM



B. FUNCTIONAL DELETION of CRD-NRG BLOCKS nAChR INDUCTION by INPUT-CM & ALTERS the NUMBER & PROFILE of SYNAPTIC nAChR CHANNELS

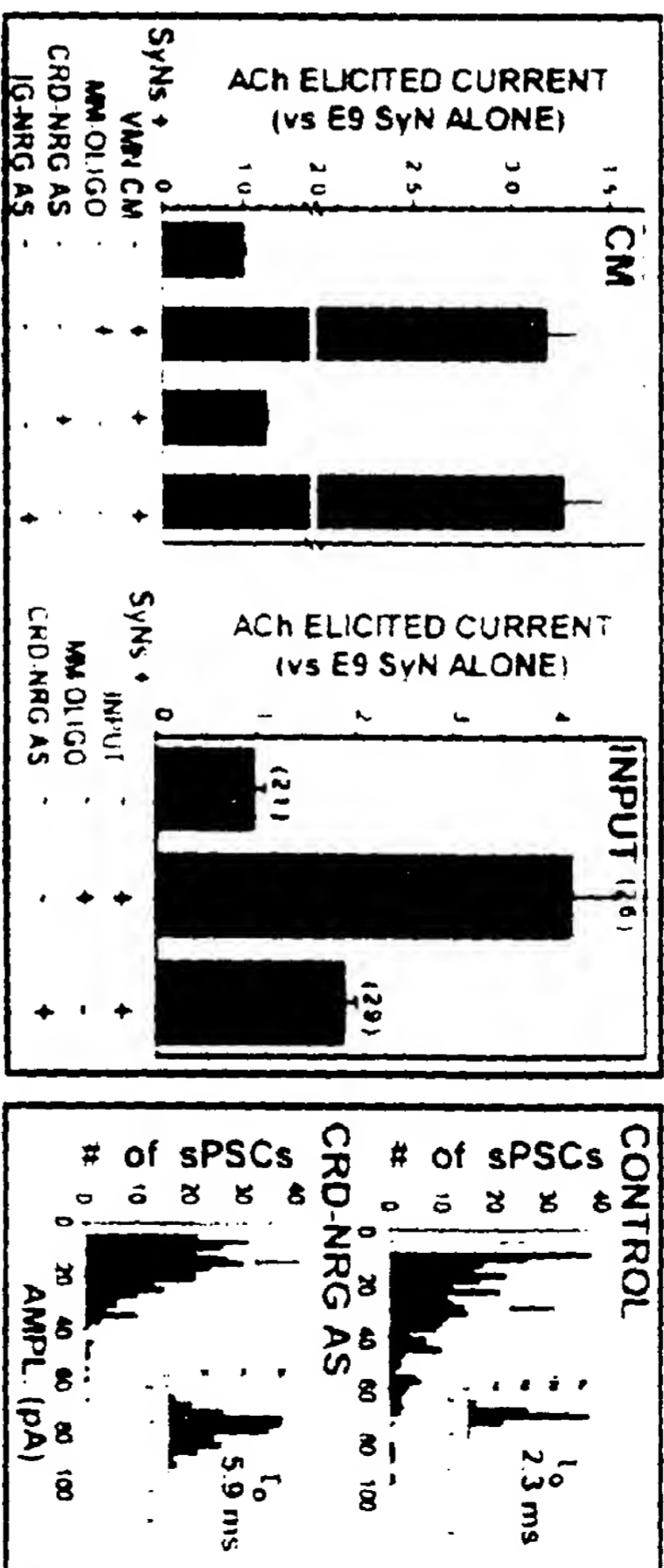
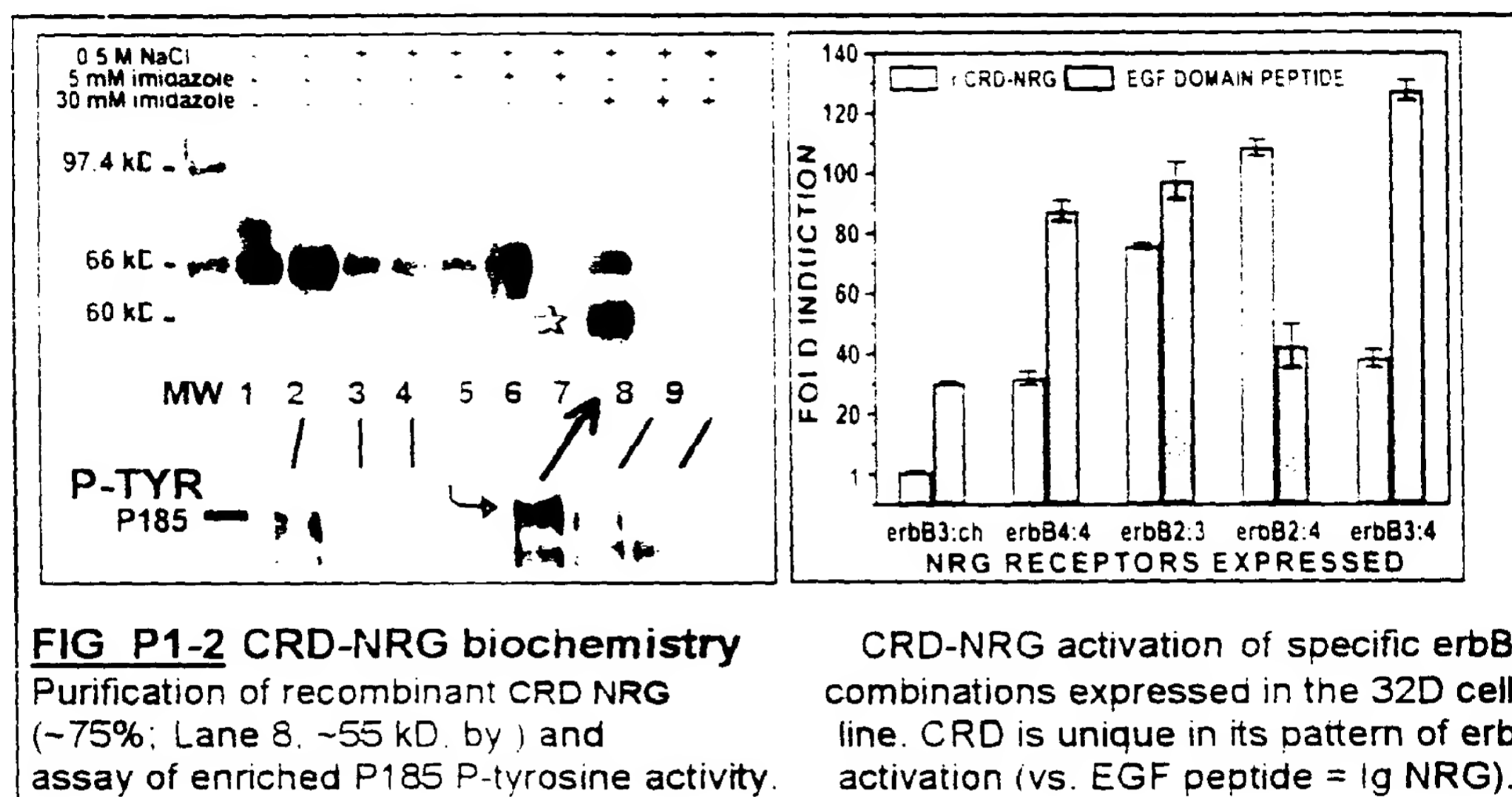


FIG. P1-1: Regulation of postsynaptic nAChRs by CRD-NRG in E9 chick sympathetic neurons.

Input-dependent induction of postsynaptic nAChRs is mimicked by CRD-NRG (A) and inhibited by CRD-NRG AS treatment (B). CRD-NRG is required for nAChR induction by input-derived soluble factors (CM) since postsynaptic nAChR induction by VMN input is strongly inhibited by CRD-NRG AS (B, Middle). Analysis of synaptic currents (sPSCs) at CRD-NRG "deleted" synapses reveals that this NRG isoform is required for the expression of the mature array of high γ , brief to nAChR channel subtypes, normally induced by VMN input (B, far right). Synaps innervated by CRD-NRG AS-treated VMN express long to, immature nAChRs, akin to those detected prior to synaptogenesis. MM = mismatch (control) oligo

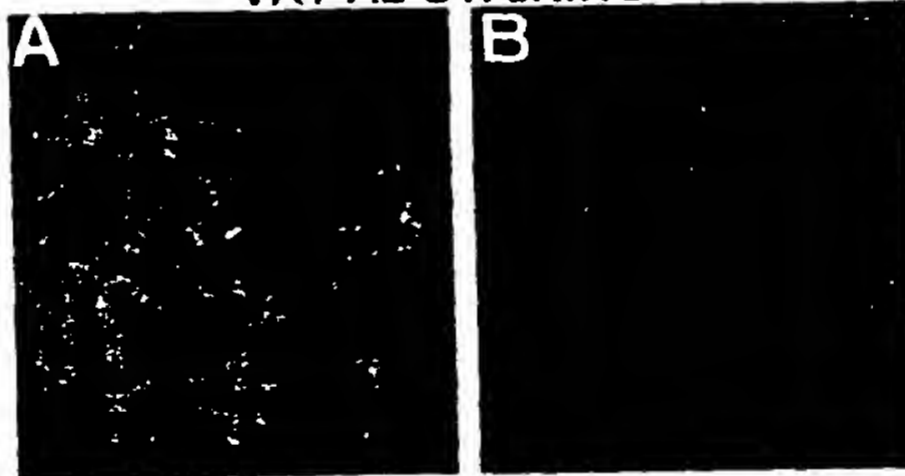
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Fig P1-3 VMN axons. within target SyMp. as seen with a VAT.Ab (-A) ,(B)
By PO VAT+ axons and the ACh-elicited I_p are diminished in CRD NRG (-/-) vs control (+/-) mice.(C, D).

CONTROL (+/-) CRD-NRG (-/-)
VAT Ab STAINING



nAChR CURRENT



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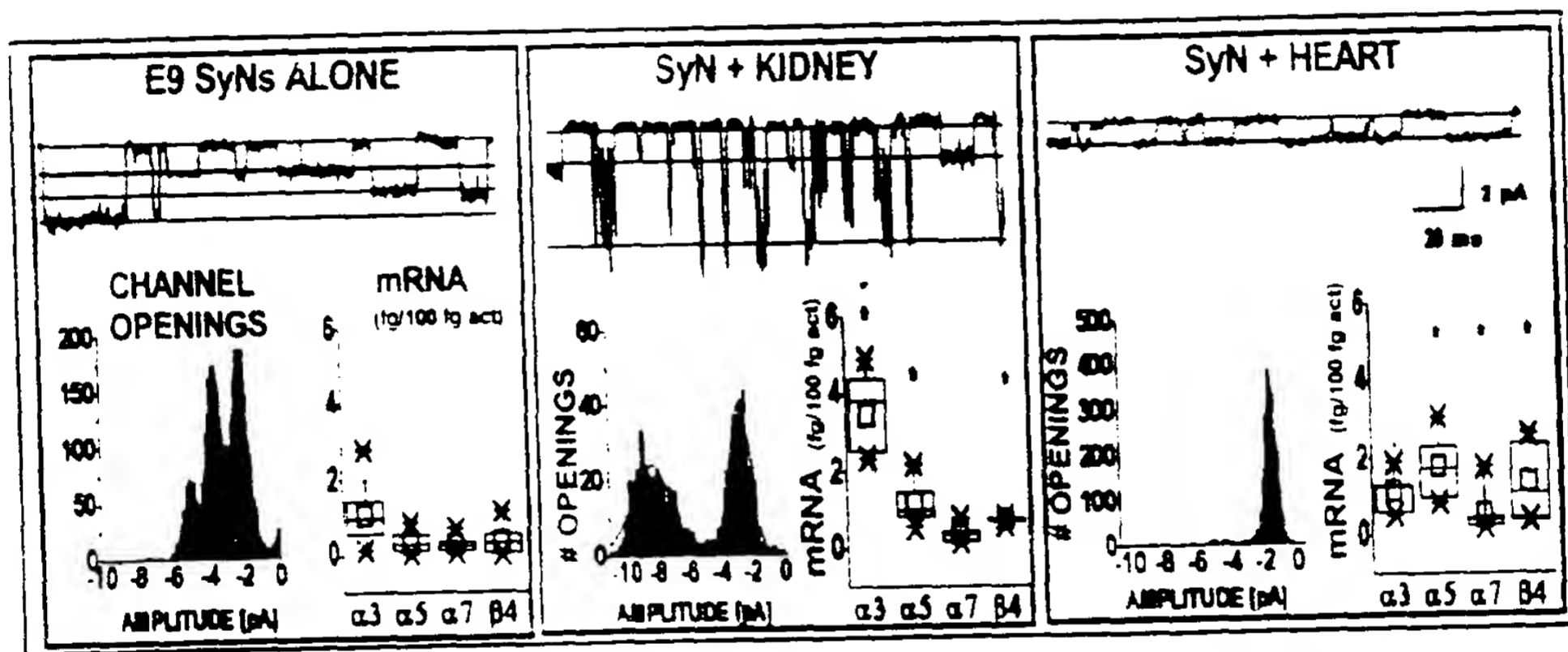


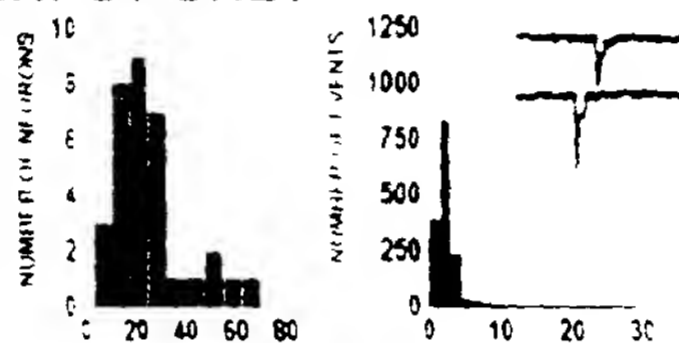
FIG. P2-1

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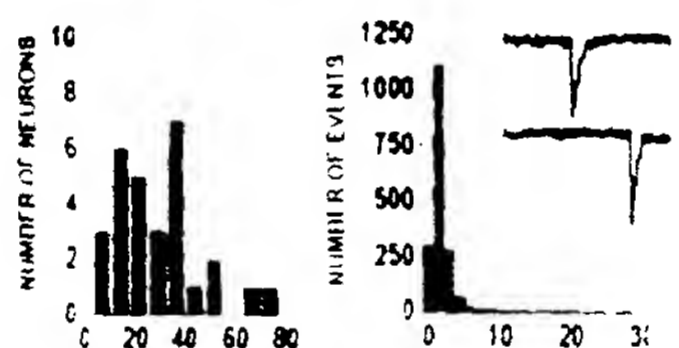
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Fig. P2-2 Target specific regulation of synaptic nAChR channels in innervated SyMp

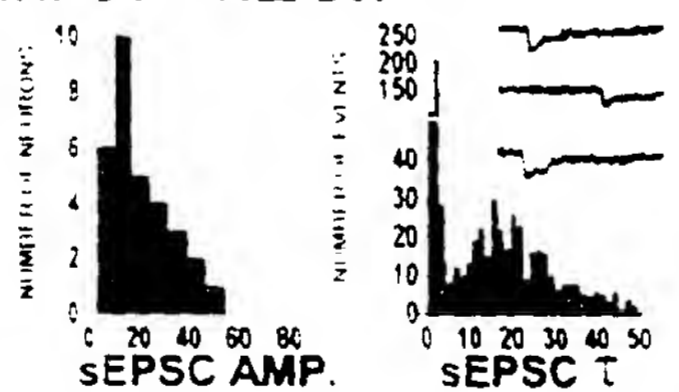
INPUT ONLY



INPUT + KIDNEY



INPUT + HEART



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| TABLE P2-1: Σ Regulation of nAChR s | | | | | | | | | |
|---|---------------------------|---------------------------------------|---------------------------|---|-------------------------------------|---|---|----------------|-----|
| γ (pS) | $(\alpha 3)_2(\beta x)_3$ | $(\alpha 3)_1(\alpha 7)_1(\beta x)_3$ | $(\alpha 3)_3(\beta x)_2$ | $(\alpha 3)_1(\alpha 5)_1(\alpha 7)_1(\beta x)_2$ | $(\alpha 3)_2(\alpha 5)_2(\beta x)$ | $(\alpha 3)_1(\alpha 5)_2(\alpha 7)_1(\beta x)$ | $(\alpha 3)_1(\alpha 5)_2(\alpha 7)_1(\beta x)$ | $\alpha 7$ | |
| γ 1 (ms) | 13.5 ± 2 | 23 ± 3 | 28 ± 4 | 38 ± 6 | 50 ± 3 | 51 ± 3 | 66 ± 7 | | |
| γ 2 (ms) | 2.1 ± 5 | 1.1 ± 0.2 | 1.7 ± 0.1 | 1.7 ± 0.1 | 3.3 ± 0.5 | 3.3 ± 0.4 | 2.5 ± 0.8 | | |
| γ 3 (ms) | 7.6 ± 1 (65%) | 7.0 ± 1 (60%) | 13 ± 0.9 | 10.8 ± 0.4 (67%) | - | 16.6 ± 3.1 (39 %) | | | |
| ABUNDANCE | | | | | | | | | |
| Early develop. | ++++ | - | ++ | - | + | - | - | - | - |
| Intermediate | +++ | + | ++ | +++ | +++ | +++ | + | + | + |
| Late develop. | - | ++ | - | +++ | +++ | +++ | +++ | ++ | ++ |
| INDUCED by... | | | | | | | | | |
| Input | - | - | - | ++ | +++ | ++ | + | + | + |
| Contact with kidney | - | - | - | + | +++ | +++ | + | +++ | +++ |
| Contact with heart | +++ | ++ | + | - | - | - | - | - | - |
| PHARMACOLOGY | | | | | | | | | |
| ACh (rel. K_{app}) | +++ | +++ | +++ | ++ | ++ | ++ | + | + | + |
| Cytisine ² | + | - | + | - | + | - | - | ND | ND |
| n-BgTx sensitivity | + | + | + | + | + | + | + | + | + |
| α -BgTx sensitivity | - | + | - | - | - | - | - | ND | ND |
| MLA sensitivity ² | - | - | - | + | - | + | + | + | + |
| DELETED by AS to: | | | | | | | | | |
| | $\alpha 3$ | $\alpha 3, \alpha 7$ | $\alpha 3$ | $\alpha 3, \alpha 5, \alpha 7$ | $\alpha 3, \alpha 5$ | $\alpha 3, \alpha 5, \alpha 7$ | $\alpha 3, \alpha 5, \alpha 7$ | $\alpha 7, ND$ | |

the number of + 's represents the relative apparent affinity for ACh (where > + 's indicates > apparent affinity)

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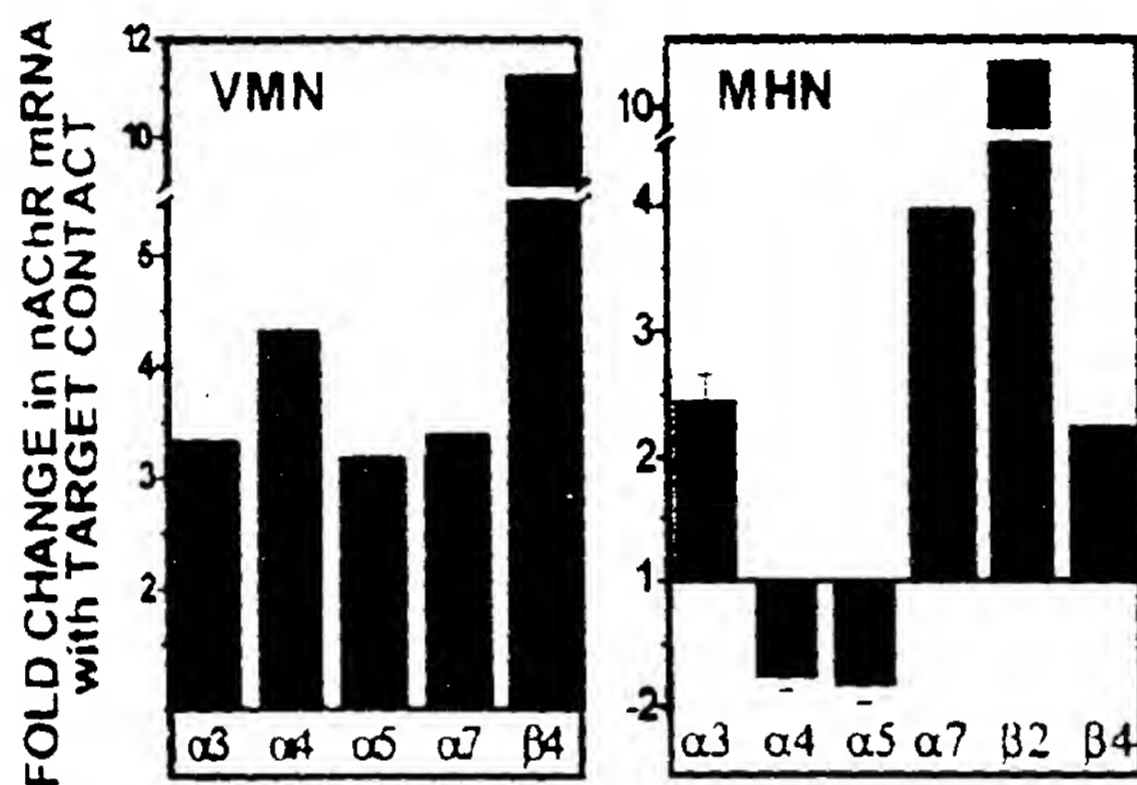
You have made it to AIM 1 FIGS AND TABLES

| Table A1-1: The profile of nAChR subunit gene expression in the visceral motor & medial habenula nuclei changes during <i>in vivo</i> synaptogenesis. | | | | | | |
|---|------------|------------|------------|------------|-----------|-----------|
| | $\alpha 3$ | $\alpha 4$ | $\alpha 5$ | $\alpha 7$ | $\beta 2$ | $\beta 4$ |
| Visc. Motor | | | | | | |
| E18 vs. P0. mouse | ↑ | ↑ | - | ↑ | ↑ | ND |
| E 9 vs. E18. chick | ND | ↑↑ | ↑ | ↑ | ↓ | ↑ |
| Med. Habenula | | | | | | |
| E16 vs. P0. mouse | ↑ | ↑ | ↑ | ↑ | ↑↑ | ND |
| E 11 vs. E17 chick | ↓ | - | ↑ | ↑↑ | ↑↑ | ↑↑ |
| qPCR assay of chick tissue extracts: mouse data from "side-by-side" in situ assays (^{FN1} , Methods). ND: not determined: - no change or low signal. | | | | | | |

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FIG A1-1: The profile of nAChR subunit gene expression in presynaptic VMN and MHN neurons is strongly regulated by interaction with neuronal targets *in vitro*.



nAChR subunit mRNA levels were assayed by qPCR of chick neurons *in vitro* - synaptic partners.. The levels of subunit mRNA in "synaptically naive" neurons is set to 1.

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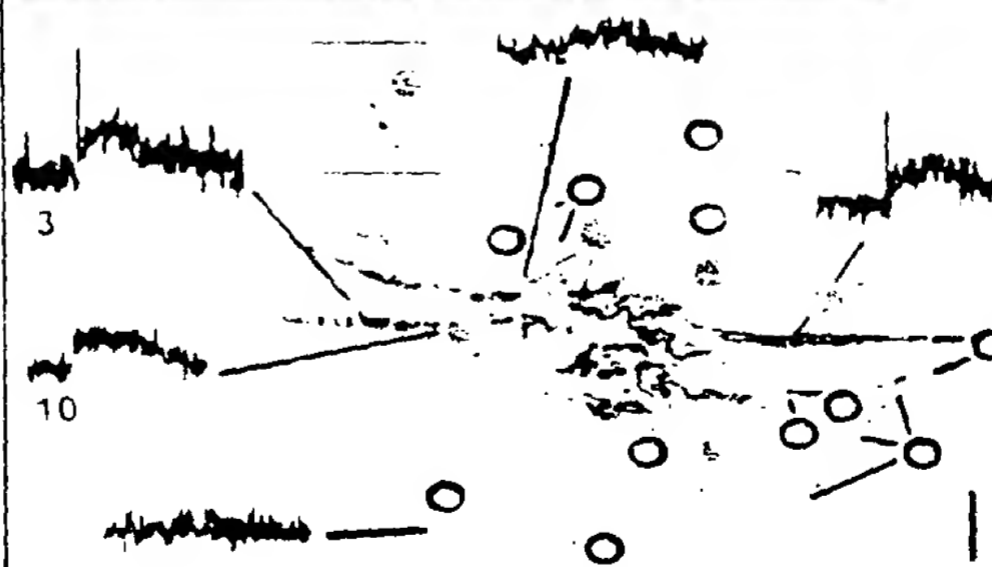
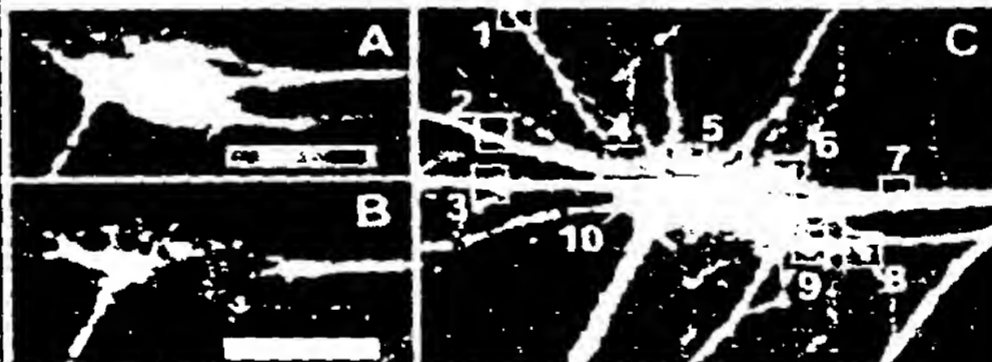
Fig A1-2 Mapping postsynaptic

"hotspots" by VC recording:

WCR and tests at 5 sites of n-n contact reveals post-synaptic hotspot areas of agonist response.



Mapping presynaptic nAChR "hotspots" by fura2-imaging of nicotine-elicited $[Ca^{2+}]$ transients. Nicotine ($1\mu M$) was applied at 25 areas eliciting increased $[Ca]$ at 10 presynaptic hotspots (red filled circles & \square in C). Blue / open circles areas tested, - after Mn quench.



Internal Mn perfusion of postsynaptic n. (A: & pseudo color purple) quenches somatic signal (B) and unveils presynaptic fibers (C: & yellow / green) calib: 0.2 fluor units x 20 secs)

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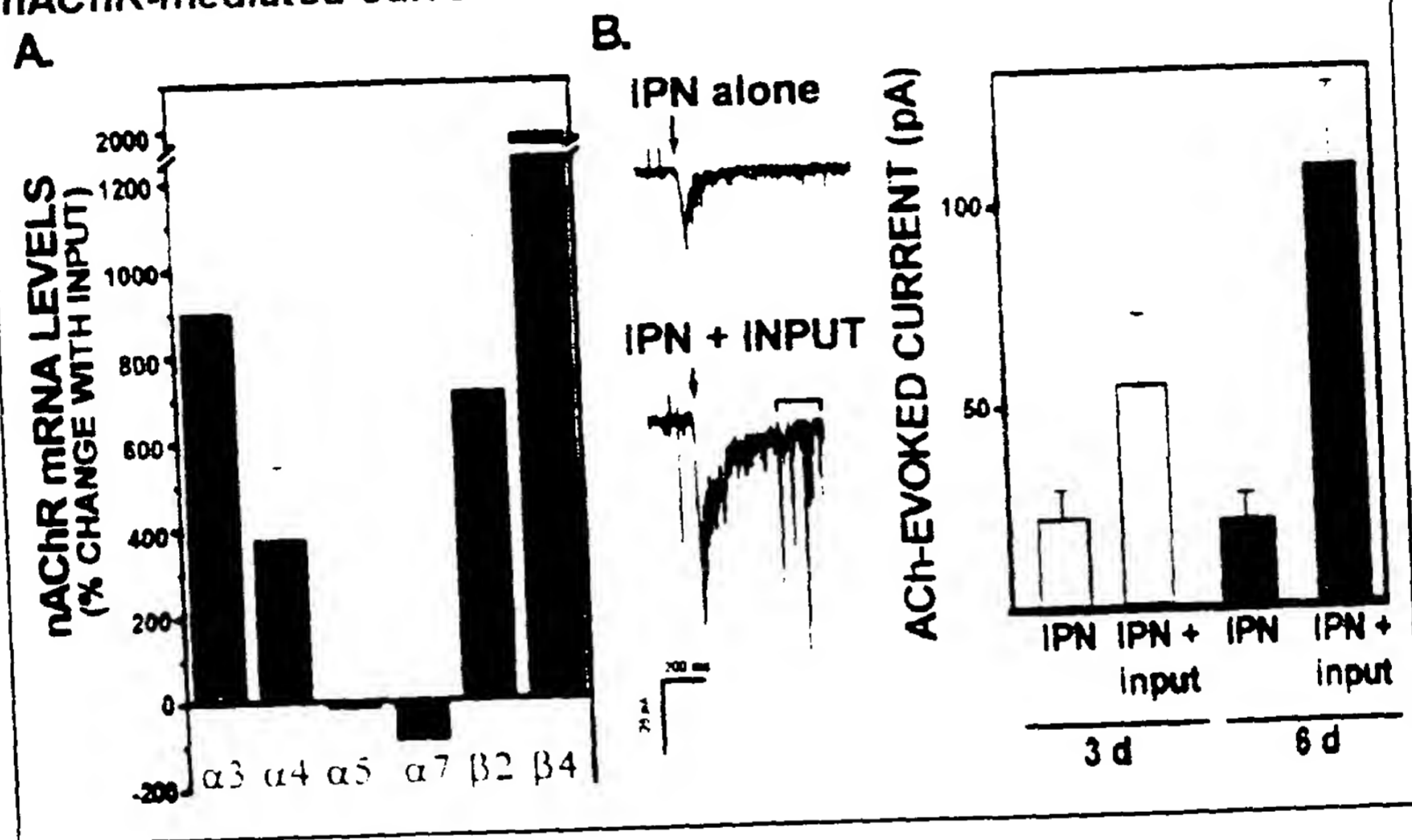
| TABLE A1-2 | AChE Fibers | $\alpha 3$ | $\alpha 4$ | $\alpha 5$ | $\alpha 7$ | $\beta 2$ |
|---------------|----------------|------------|------------|------------|------------|-----------|
| IPN | | | | | | |
| E16 | + | - | - | + | - | ++ |
| E18/P0 | +++ | +/- | + | ++ | +/- | ++ |
| P7 | +++ | ++ | ++ | +++ | ++ | + |
| AMYG | | | | | | |
| E16 | - | - | - | - | - | - |
| E18/P0 | +(gc) | +/- | ++ | +/- | + | + / ++ |
| P7 | +++ | ND | +++ | + | +++ | ++ |

"Amygdala" refers to 2 major cholinceptive subregions: the basolateral nucleus (BLA), and the Nucleus of the lateral olfactory tract nuclei (NLOT). (gc)= growth cone tipped AChE + fibers. ND= not determined.

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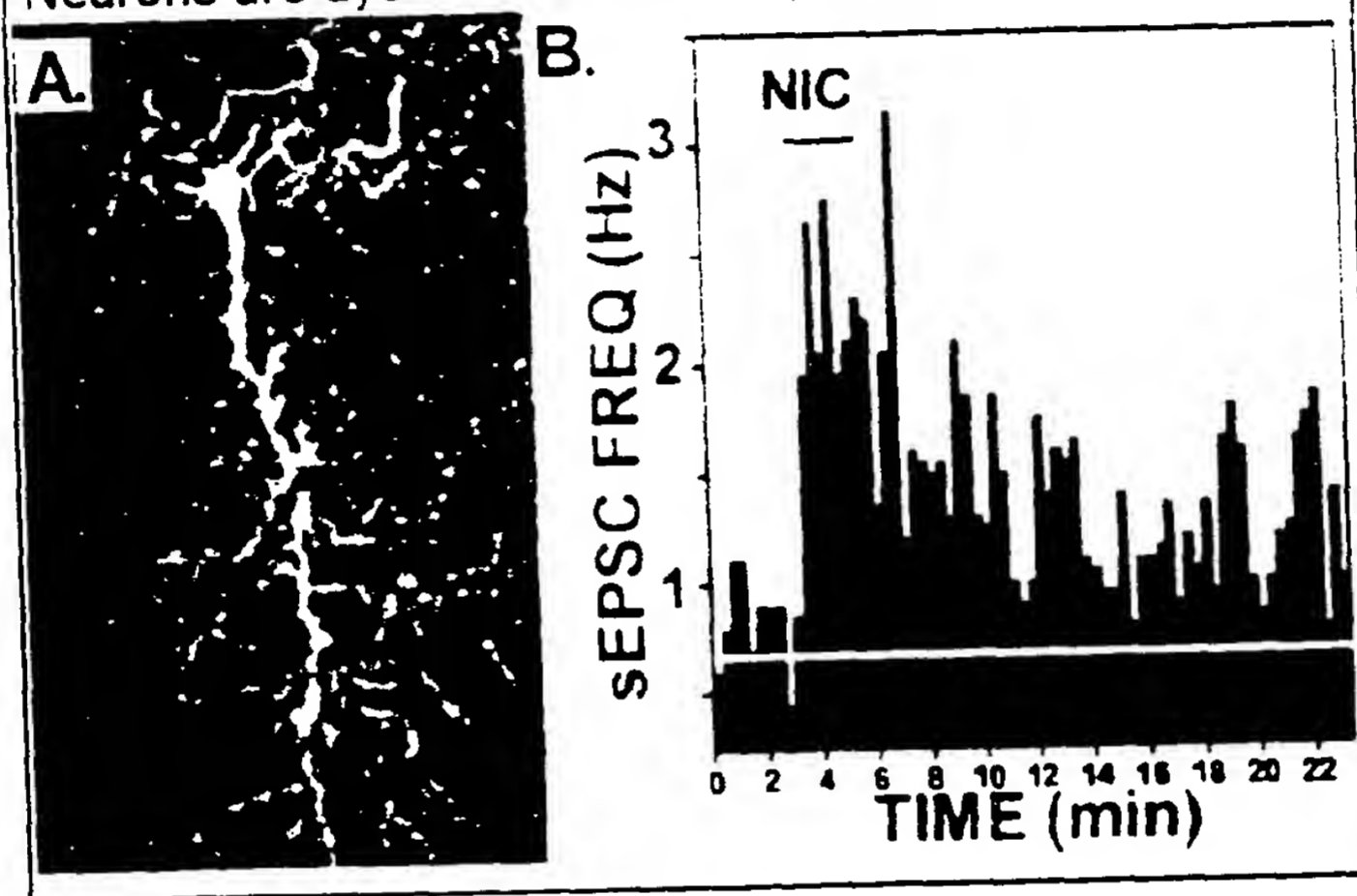
Fig A1-3: *In vitro* innervation of IPN by MHN alters the profile of nAChR subunits expressed and increases the magnitude of nAChR-mediated currents



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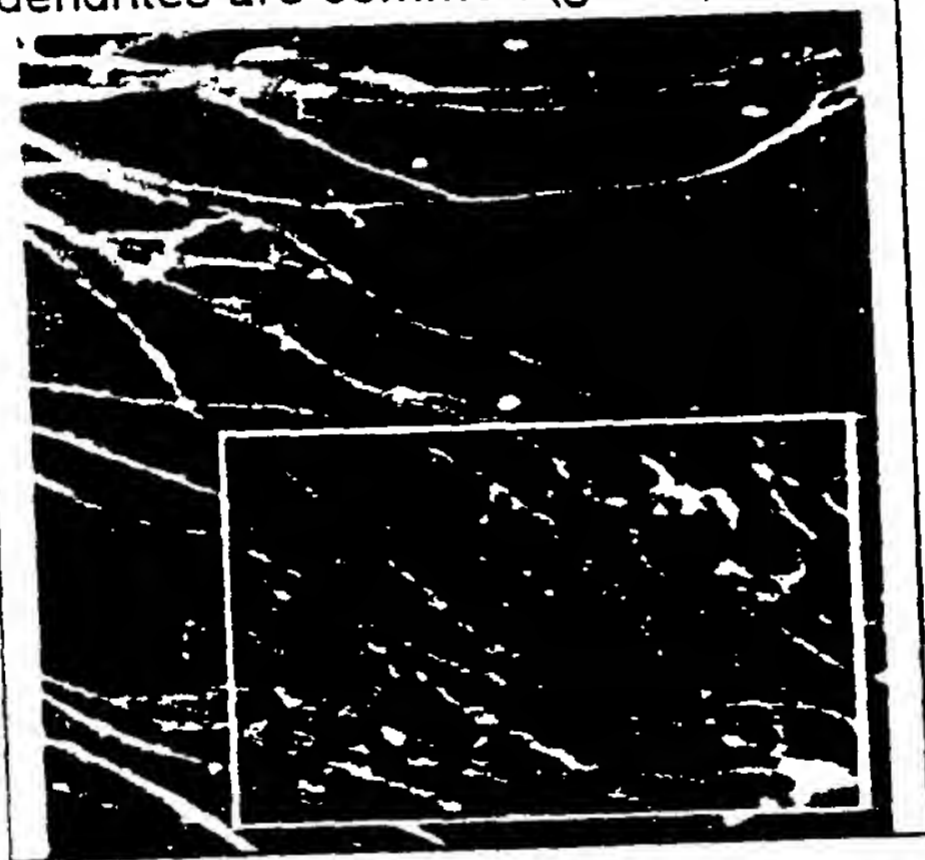
Fig A1-5: Nicotine induces robust synaptic facilitation: slice-patch recording from P0 mouse IPN (B). Neurons are dye filled for subsequent re-location (A)



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Fig A1-4: Cholinergic fibers exit mouse MHN micro-explants (VAT+: red). Contacts with IPN MAP + dendrites are common (green).



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| TABLE A2-1 | $\alpha 3$ | $\alpha 4$ | $\alpha 5$ | $\alpha 7$ | $\beta 2$ | $\beta 4$ |
|---|-----------------------------|--------------------------------------|------------------------------|--|--|--|
| VMNs <i>In vivo</i> (mouse) DEV. Δ 's; E16 vs. P0 CRD(-/-) vs. CONT (chick) <i>in vitro</i> | \uparrow ND | \uparrow $\downarrow\downarrow$ | - - | \uparrow \downarrow | \uparrow \downarrow | ND ND |
| Δ with target Δ with CRD NRG | \uparrow $\downarrow?$ | $\uparrow\uparrow$ \uparrow | \uparrow no | \uparrow \uparrow | ND ND | $\uparrow\uparrow$ $\uparrow\uparrow$ |
| MHN <i>In vivo</i> DEV. Δ 's CRD(-/-) vs. CONT <i>in vitro</i> | \uparrow no Δ | \uparrow $\downarrow?$ | \uparrow \downarrow | \uparrow \downarrow | $\uparrow\uparrow$ $\downarrow?$ | $\uparrow\uparrow$ ND |
| Δ with target Δ with CRD NRG | \uparrow \uparrow | \downarrow \uparrow | \downarrow \downarrow | $\uparrow\uparrow$ $\uparrow\uparrow\uparrow$ | $\uparrow\uparrow\uparrow$ $\uparrow\uparrow\uparrow$ | \uparrow $\uparrow\uparrow$ |
| IPN <i>In vivo</i> DEV. Δ 's CRD(-/-) vs. CONT <i>in vitro</i> | $\uparrow\uparrow$ "ND" | \uparrow \downarrow | \uparrow \downarrow | \downarrow no | \uparrow no Δ | $\uparrow\uparrow$ ND |
| Δ with input Δ with CRD NRG | \uparrow \uparrow | \uparrow ND | $\uparrow?$ $\uparrow?$ | \downarrow $\downarrow\downarrow$ | $\uparrow\uparrow$ ND | $\uparrow\uparrow$ -/? \uparrow |
| AMYGD <i>In vivo</i> Δ DEV (P0 vs. P7) Δ in CRD(-/-), P0 m. E16 mouse <i>in vitro</i> | ND ND | \uparrow \downarrow | $\uparrow?$ $\downarrow?$ | $\uparrow\uparrow\uparrow$ $\downarrow?$ | no Δ no Δ | ND ND |
| Δ with input | $\sim\uparrow$ | $\sim\uparrow$ | $\sim\uparrow$ | $\uparrow\uparrow$ | no Δ | ND |

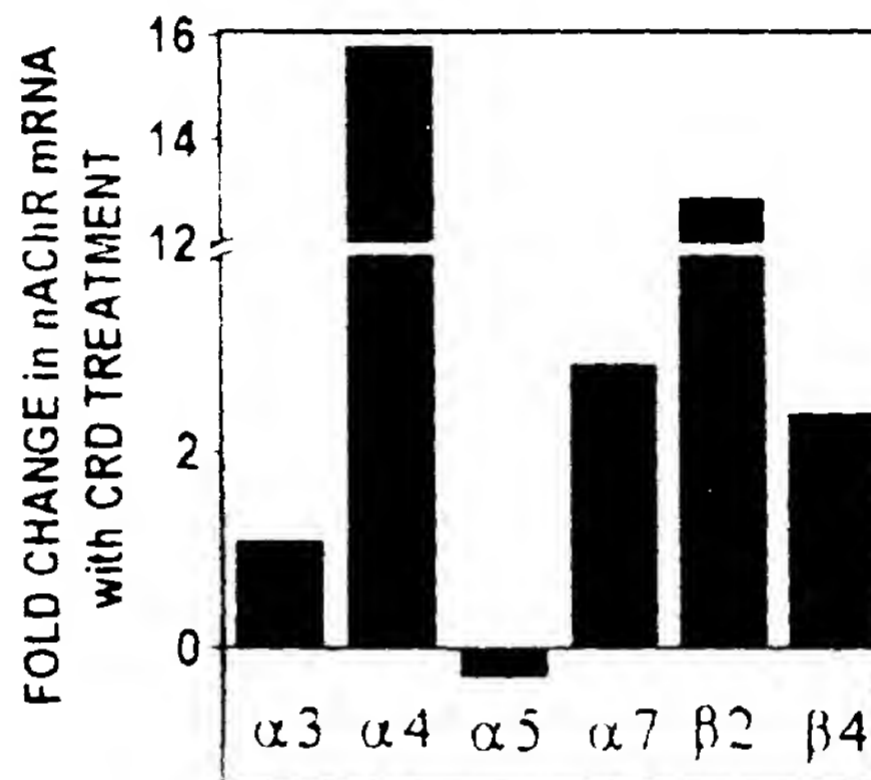
Mouse" *in vivo*" data refers to preliminary *in situ* analyses. All *in vitro* data refer to qPCR assays. no Δ : no change in subunit levels. ND= not determined ; - or ?; measurement uncertainty due to low "n" or low levels of expression. Also see Fig A2-2;

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Fig A2-1: Recombinant CRD-NRG alters profile of nAChR subunit genes expressed in chick MHN neurons

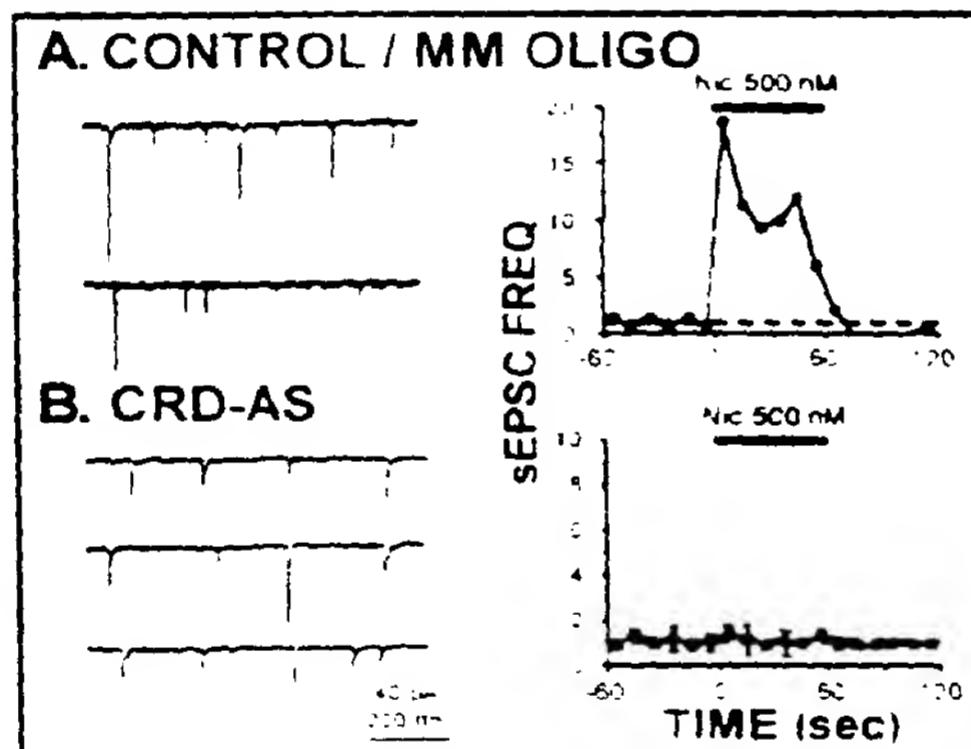


nAChR subunit mRNA levels were assayed by qPCR of chick MHN neurons *in vitro*, treated (24 hrs) with rCRD-NRG or mock. The levels of subunit mRNA in mock treated neurons is set to 1.

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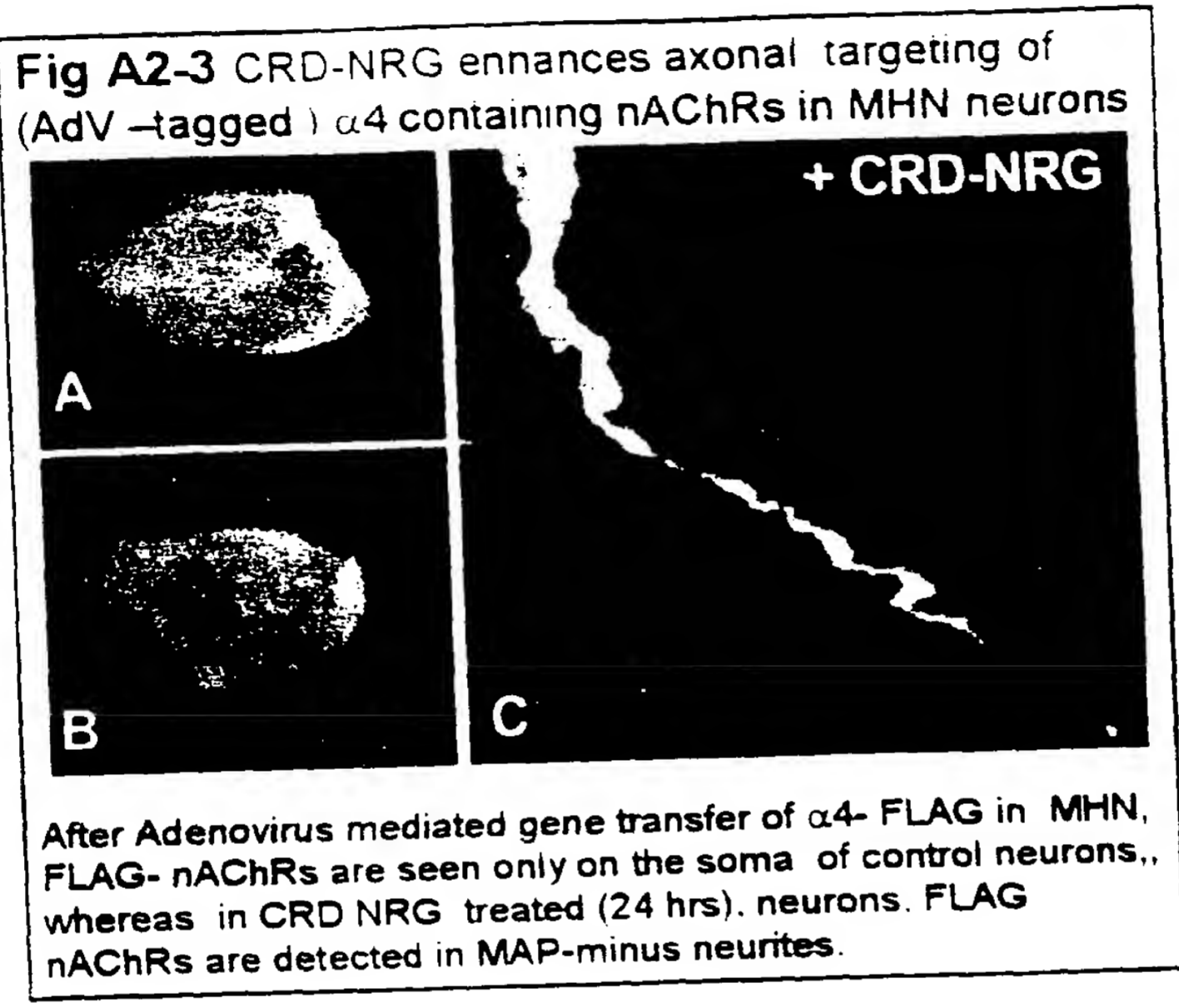
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Fig A2-2 CRD-NRG signaling may be required for expression &/or targeting of presynaptic nAChRs in CNS neurons



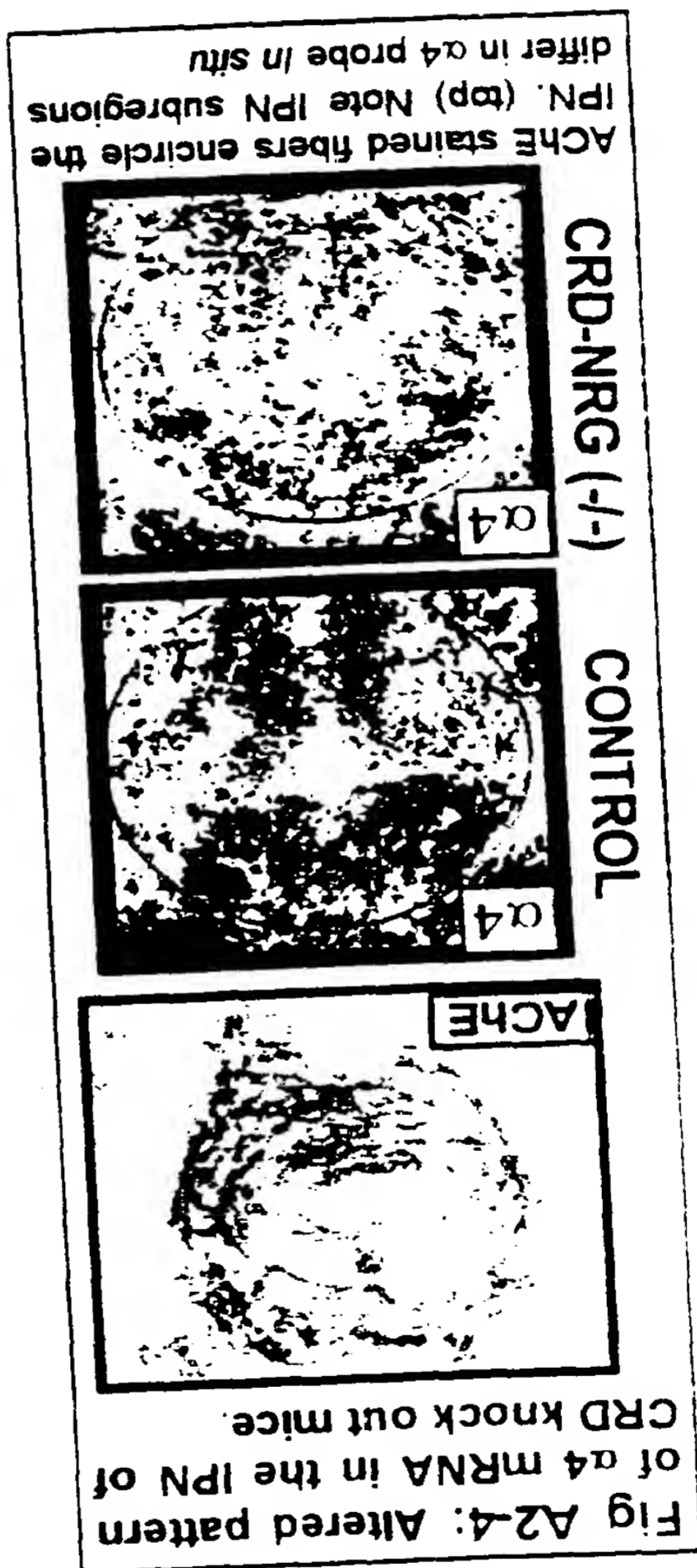
(A) Control: presynaptic nAChRs are present at VMN - SyMp synapses, as detected by increased sEPSC frequency (synaptic facilitation) with applied nicotine. (B) Treatment of VMNs with CRD-NRG AS- (48 hrs) blocks nicotine-induced facilitation, although baseline synaptic activity (sEPSC freq. without nicotine) is unaffected. MM = mismatch (control) oligo

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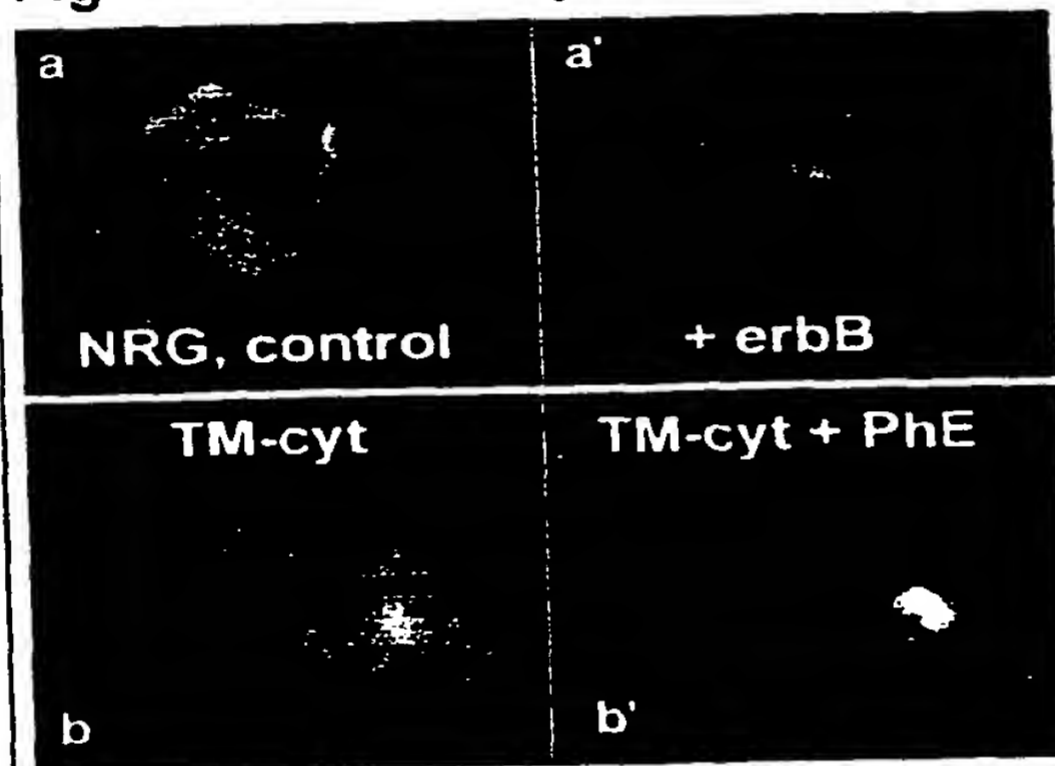
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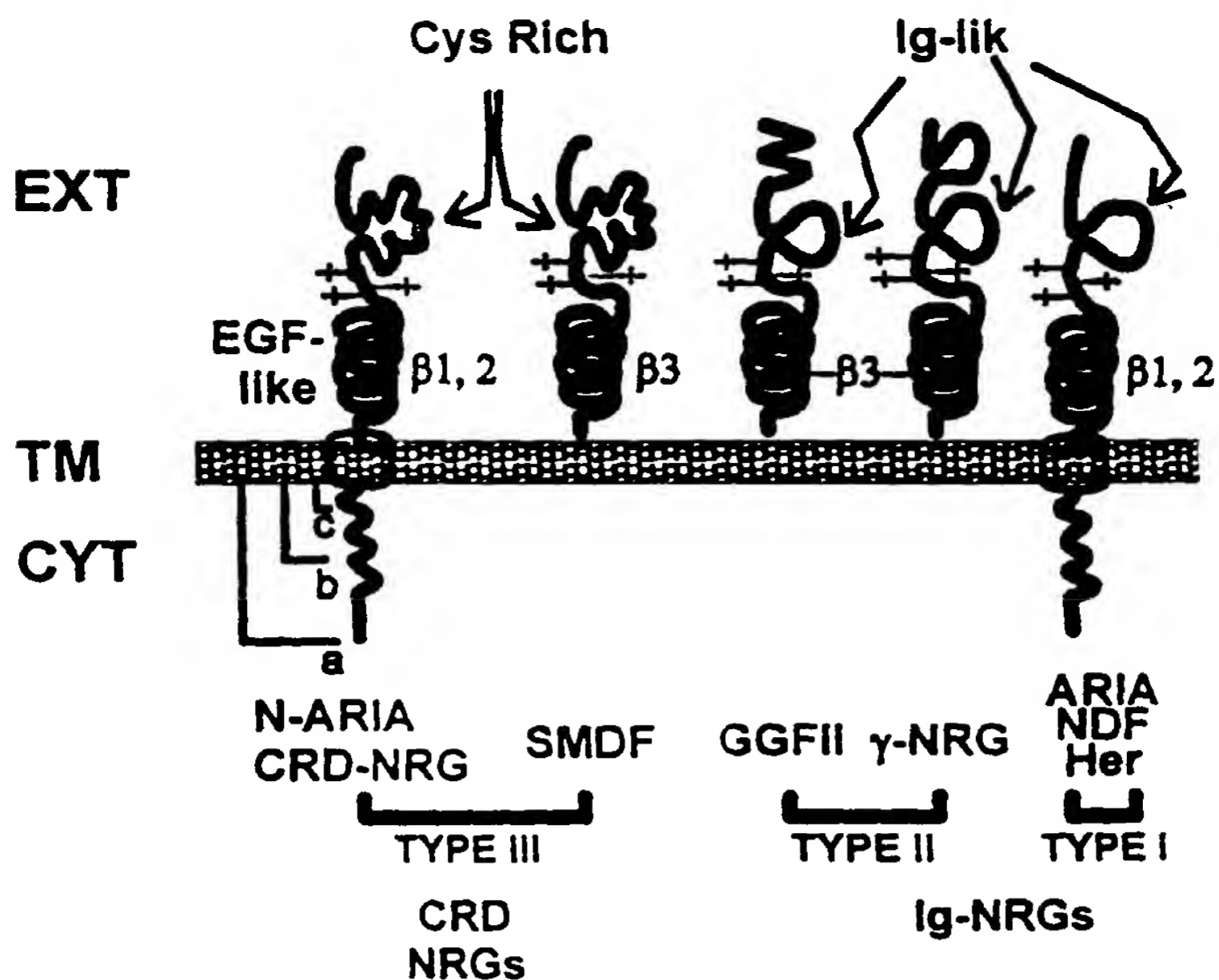
Fig A3-1 Nuclear targeting of NRG-CYT



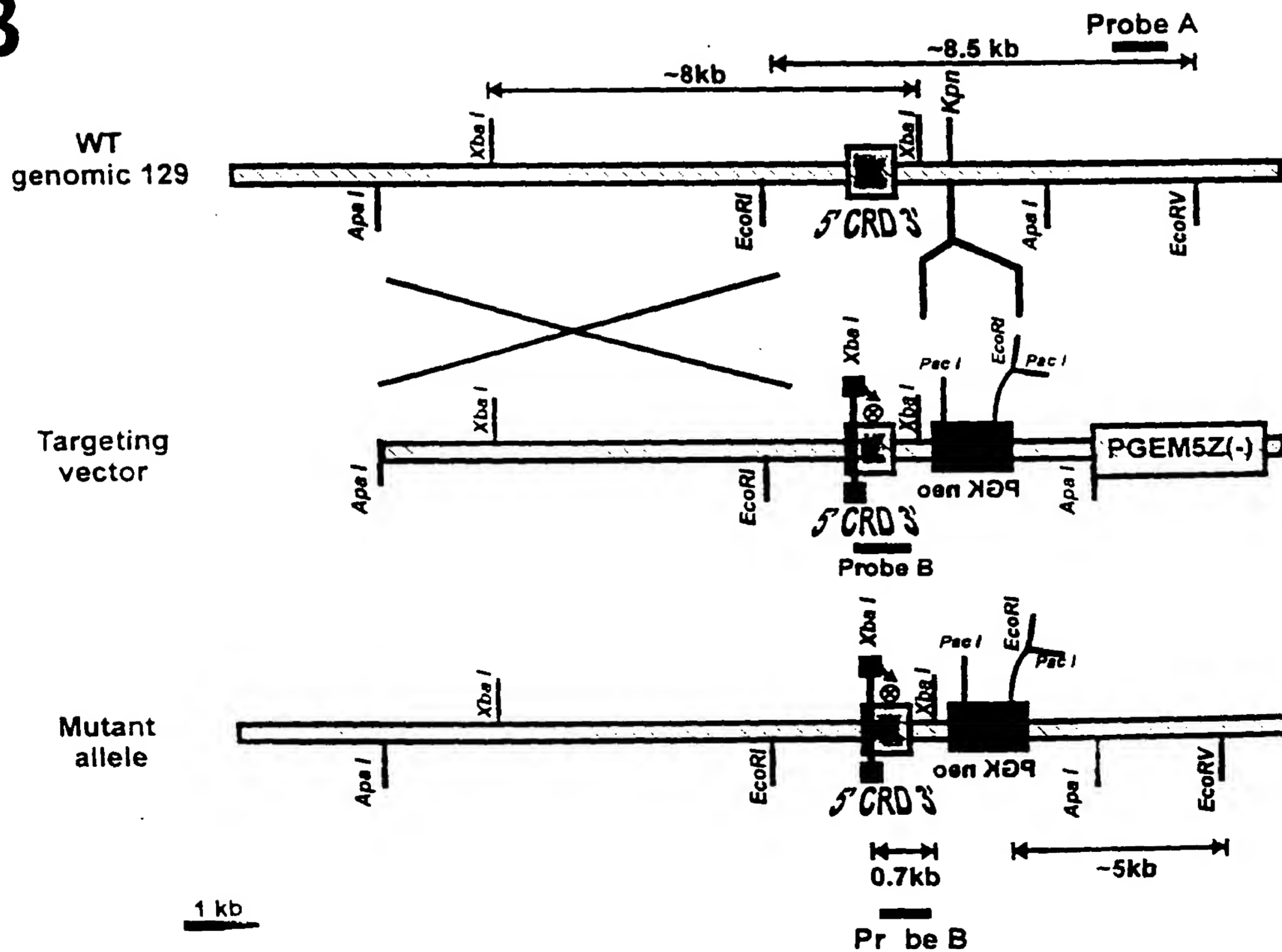
Addition of erbB expressing cells (a, a') or activation of PKC (b, b') induces nuclear targeting of the cytoplasmic (CYT) domain from a full length NRG or a NRG transmembrane (TM) + CYT chimera

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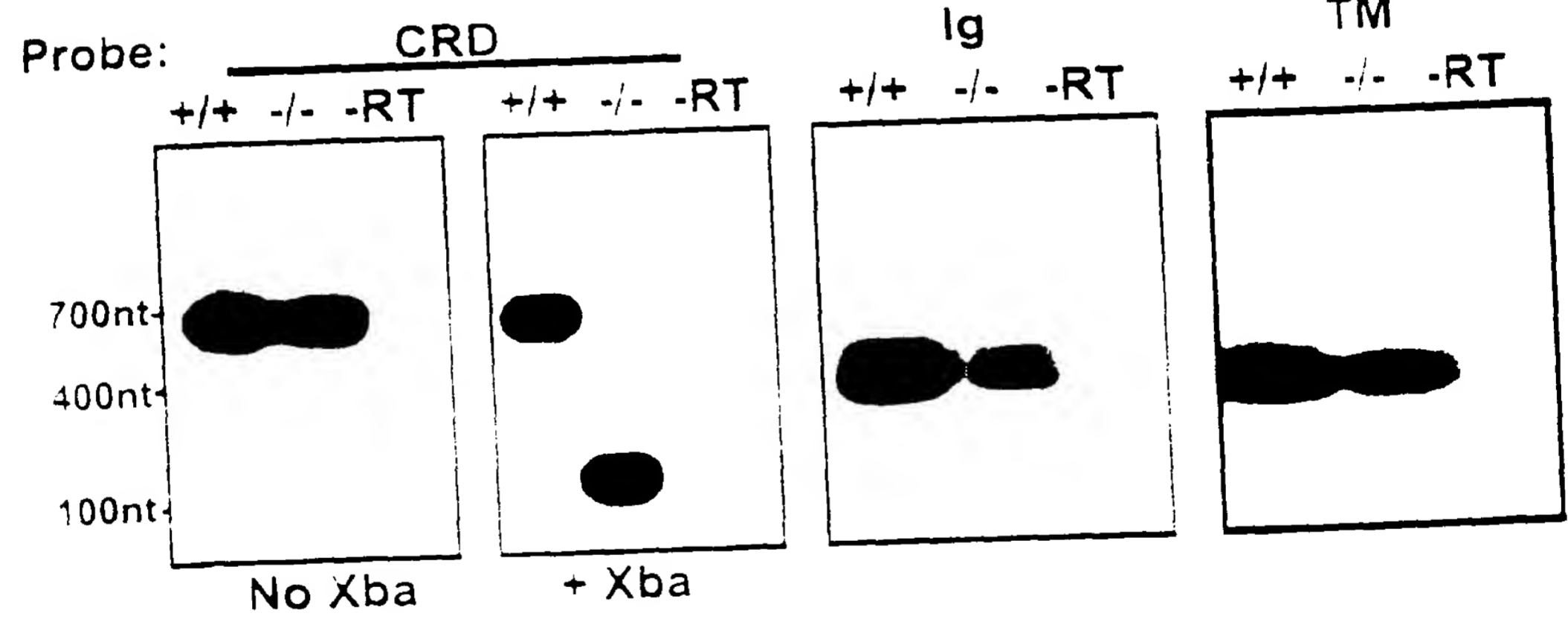
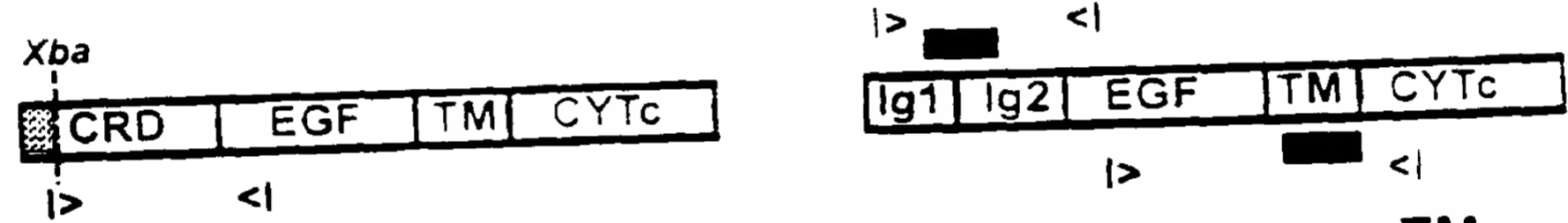
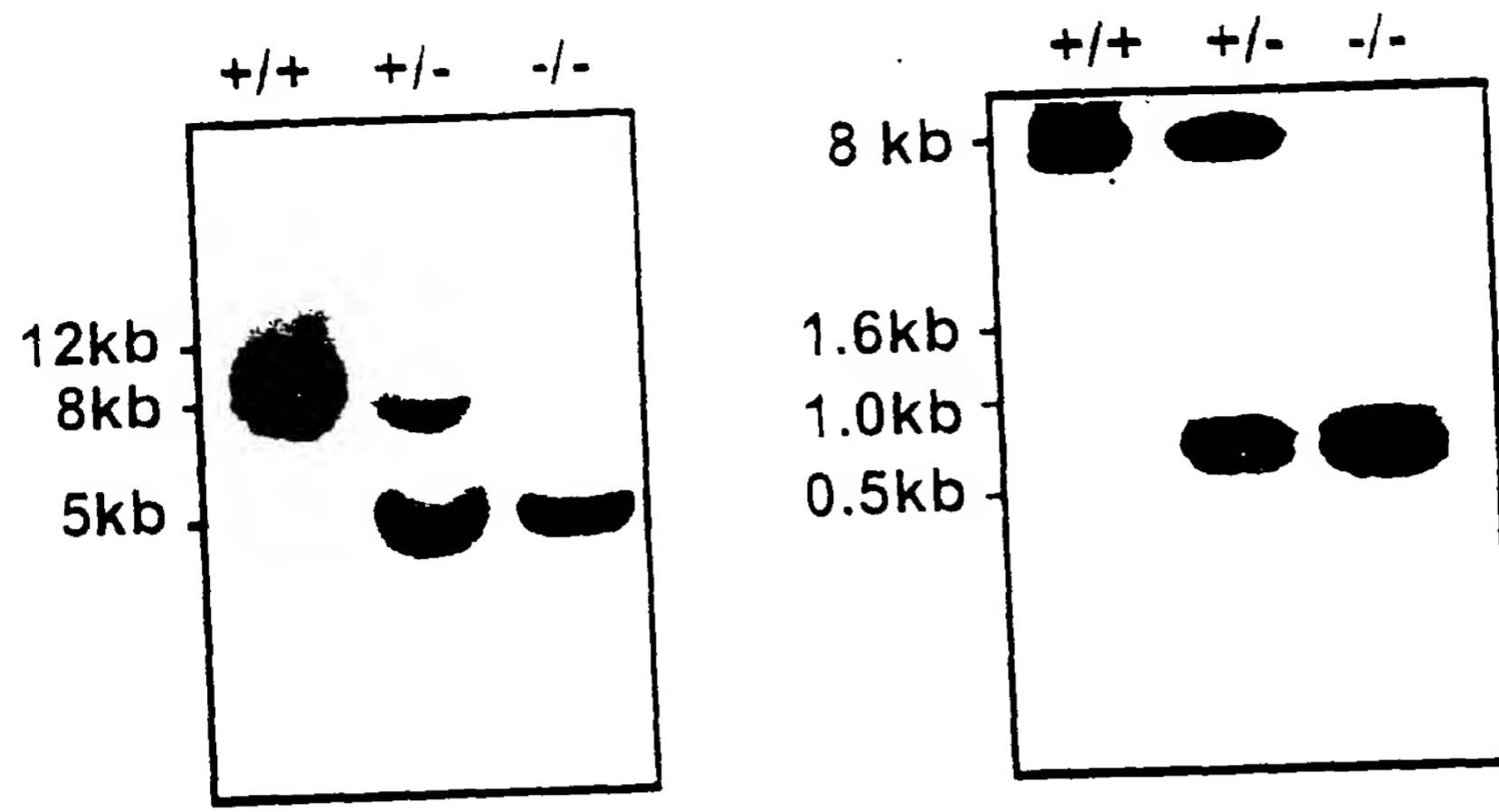
A



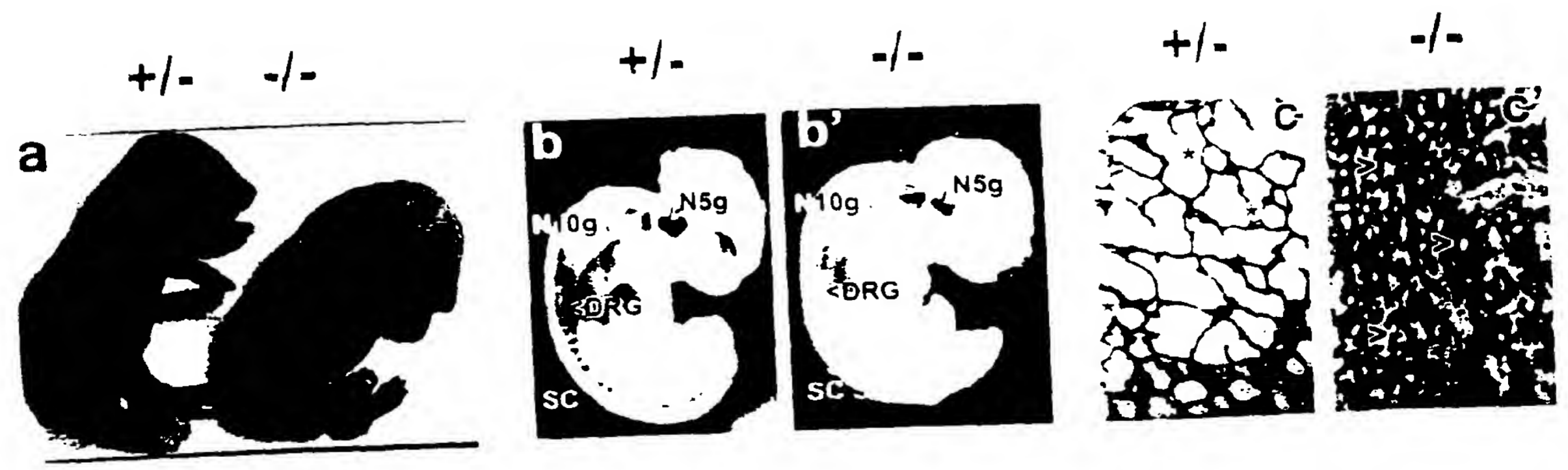
B



C



E



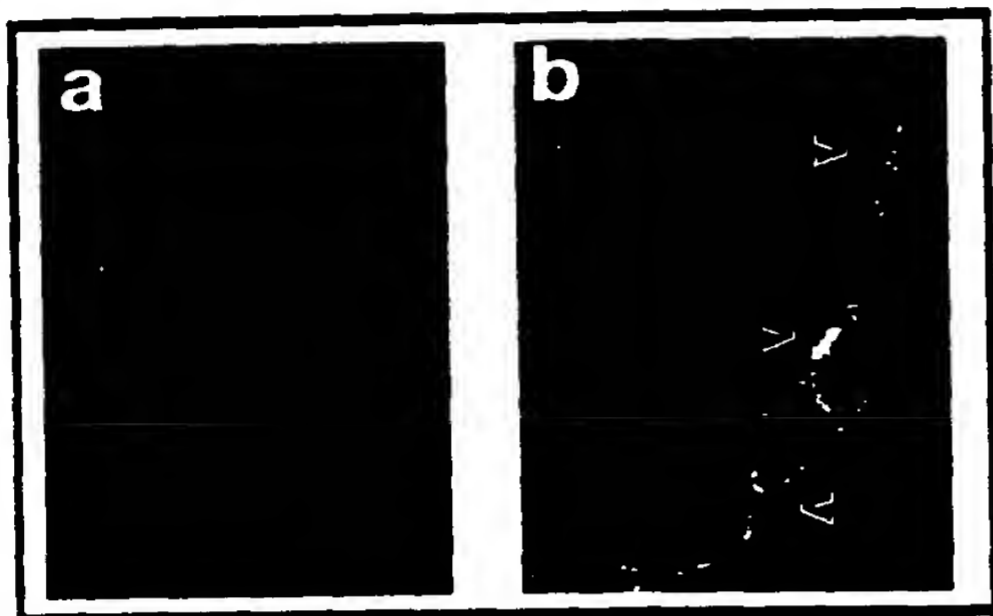
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A

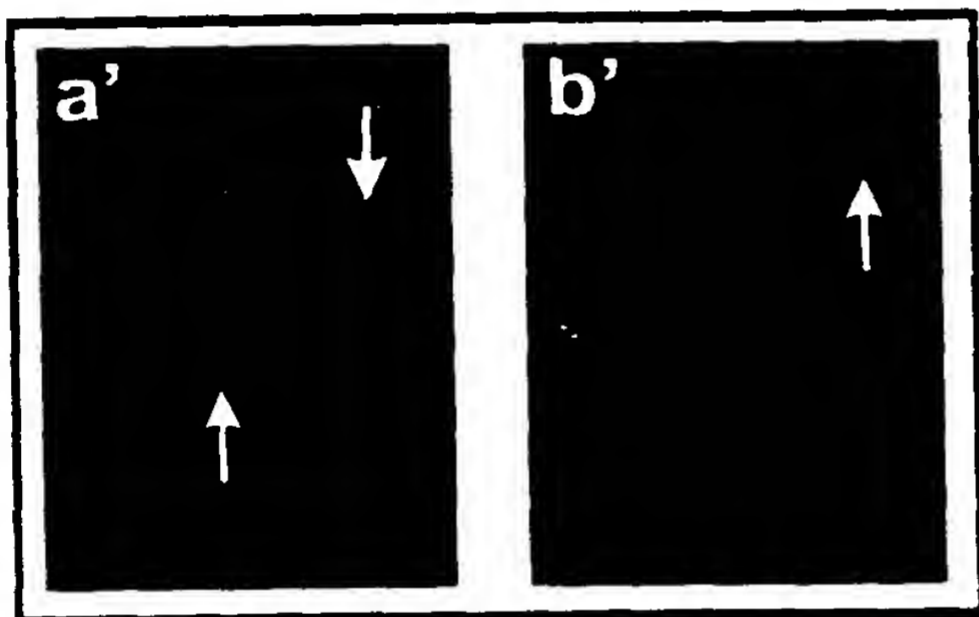
FIGURE 2A Wolpowitz et al

P0

CONTROL



CRD-NRG (-/-)



NF

+ α BgTx



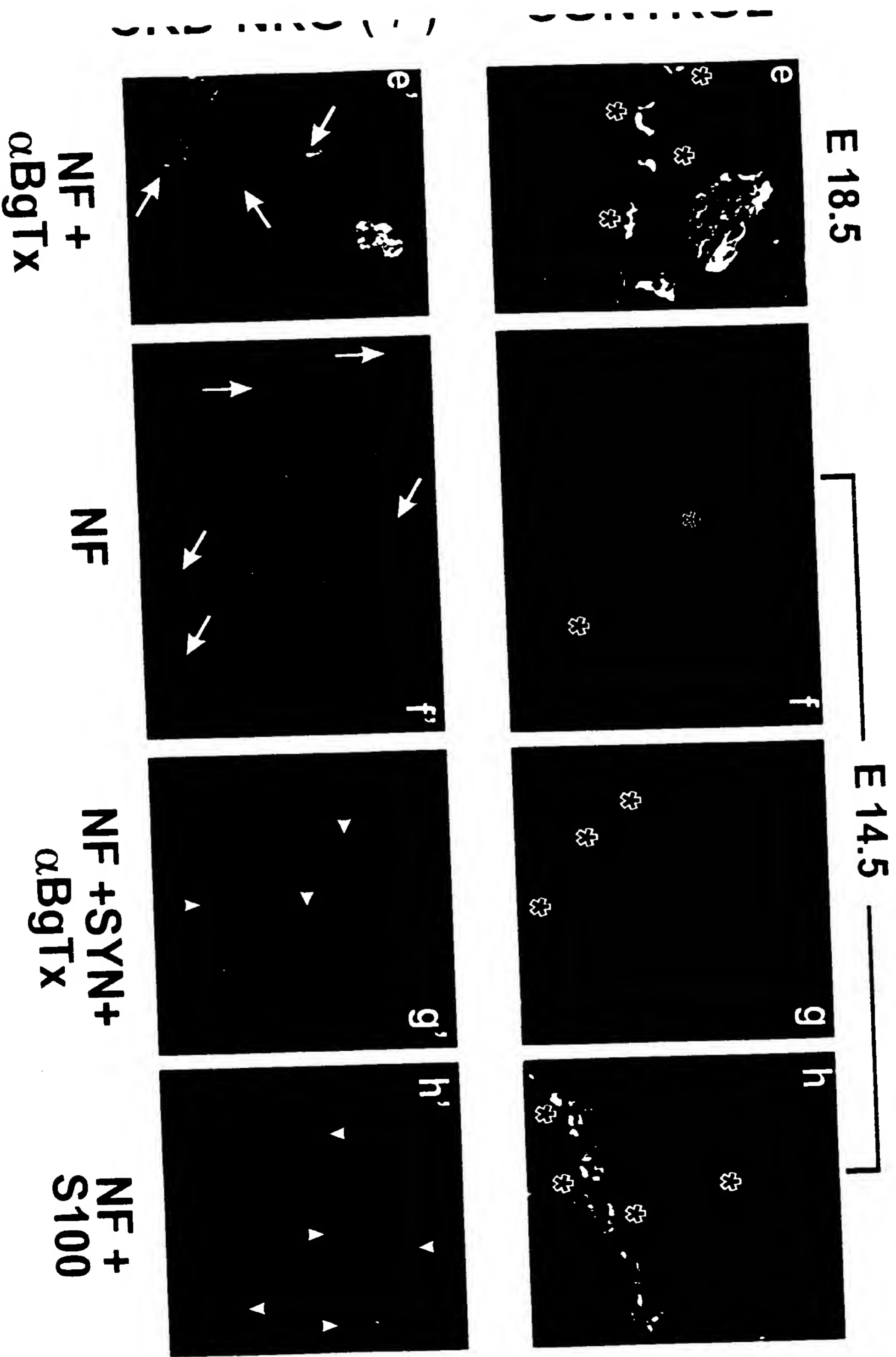
AChE



H&E

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FIGURE 2B Wolpowitz et al

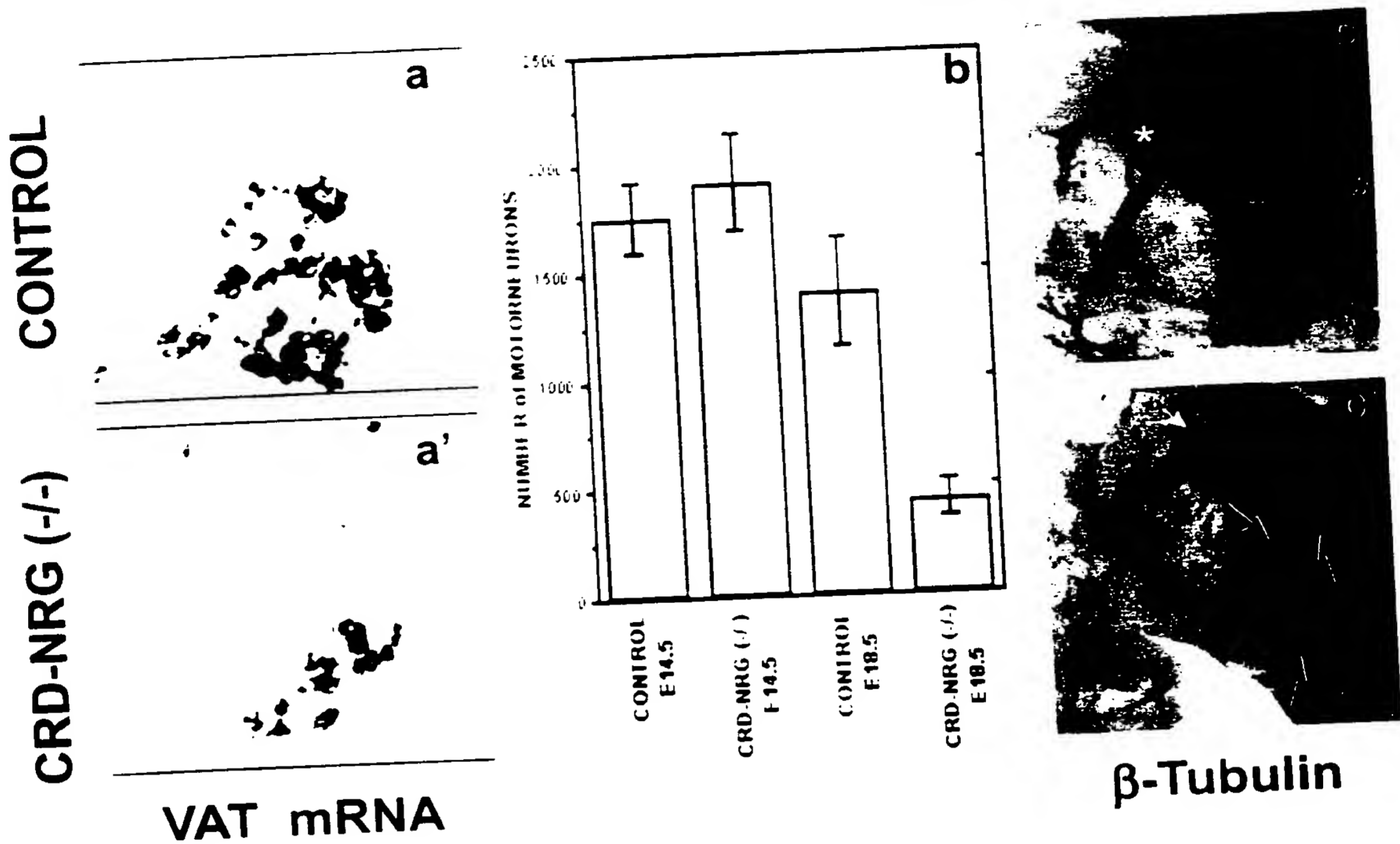


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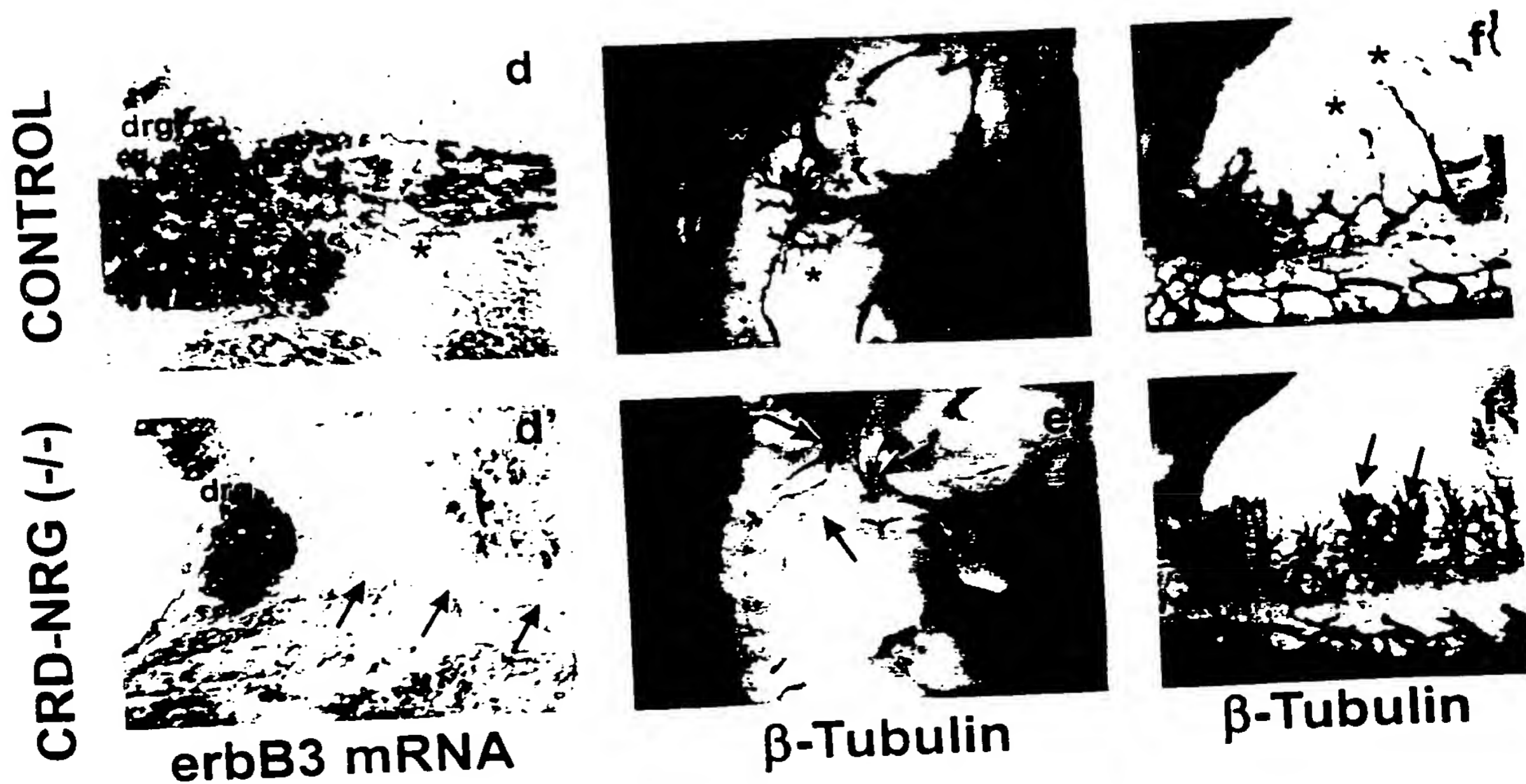
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FIGURE 3(A,B) Wolpowitz et al

A



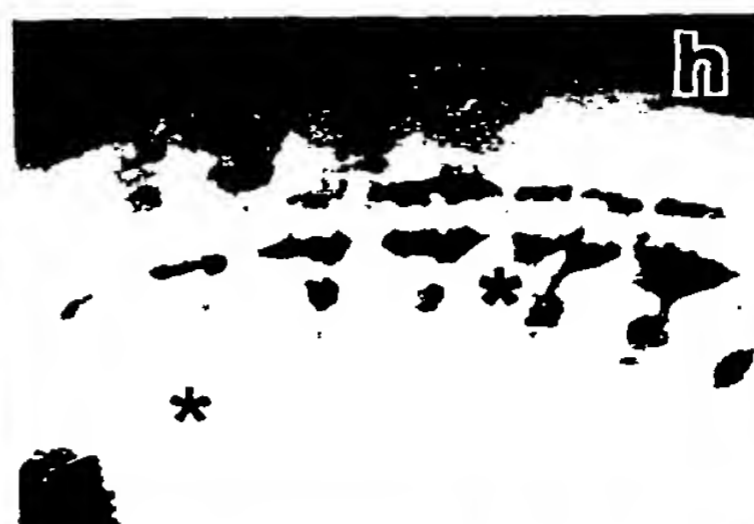
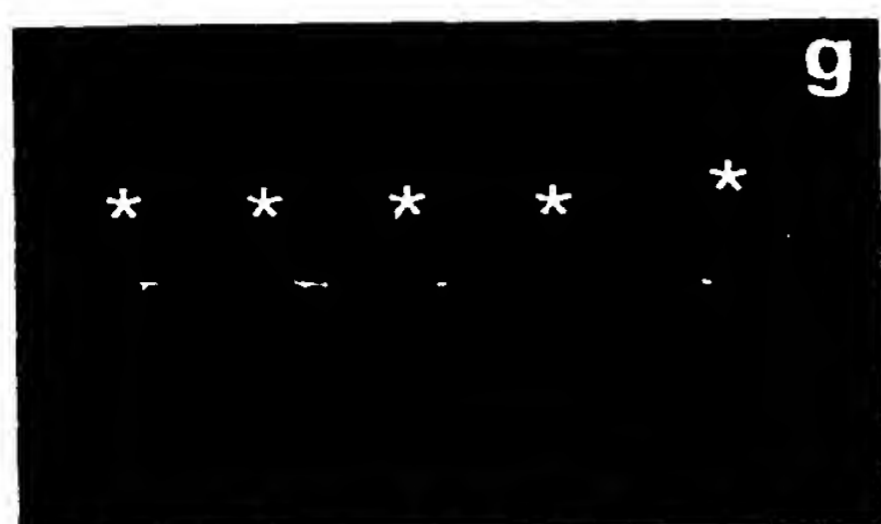
B



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C

CRD-NRG (-/-) CONTROL



VAT Ab

erbB3
mRNA

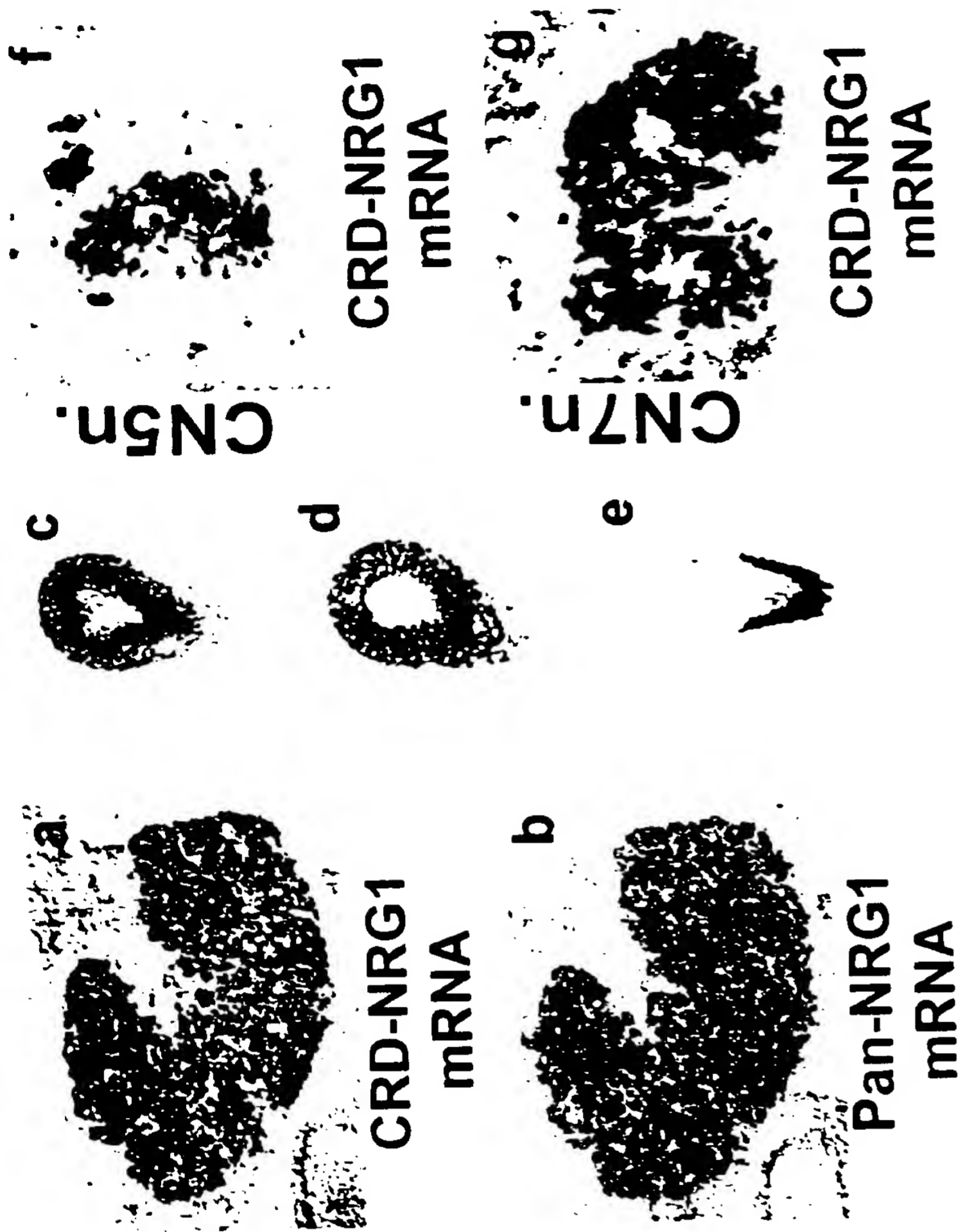
VAT +
s100

FIGURE 3(C) Wolpowitz et al

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CN5-GANGLION



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B

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